

nature

24 May 1979

A select committee to be kept

WITHIN a period of weeks rather than months the UK House of Commons is likely to start discussing changes in procedure, and high on the list of priorities will be a re-organisation of the select committee structure. There is a wide measure of support in parliament for the re-aligning of committees along ministerial lines, and were this to come about the Select Committee on Science and Technology would be disbanded; scientific issues would then be the concern of, at the least, the select committees on agriculture; defence; education, science and the arts; energy; environment; industry; and social services. Professors Ziman and Denbigh recently expressed the view of the Council for Science and Society that this would be a retrograde step (10 May, p 100).

This is a view which will be widely shared by scientists and technologists. The present committee has come in for its fair share of criticism from us and others in the past, but at least it has provided a fairly effective way by which the communities of science and technology could make contact with parliament, in an almost totally non-partisan way, on matters of concern. In none of the proposed new

committees will scientists find their interests and worries given anything like the same attention—least of all in the education, science and arts committee, where education will surely swamp everything else.

The scientific and technological community, almost by its very nature, has never had more than a handful of its number in the Commons. During its time, however, the select committee has turned up several non-scientists who have taken an active and highly intelligent interest in scientific matters; in the new order there is unlikely to be any incentive for such people to come forward.

Finally, the assumption made in the new scheme is that the way the government divides its ministerial responsibilities is the way that major national issues divide. This is simply not true in the scientific and technological field. Who would look at genetic manipulation? The impact of microprocessors? Support for innovation? Scientific manpower problems? Training for the engineering profession? Climatic change? These are all matters in which parliamentarians ought to be informed. And none of them falls neatly into one department's remit. □

Elitist patronage—and rightly so

FIVE years ago the German pharmaceutical company C. H. Boehringer Sohn of Ingelheim invited a group of biologists, medical researchers, and philosophers to spend a few days together in a well-appointed Schloss far from the pressures of colleagues, students and telephones. The group brooded on the question of creativity, and a report of the deliberations was later published (*The creative process in science and medicine* edited by Hans Krebs and Julian Shelley; Excerpta Medica).

The indefatigable Dr Shelley, Boehringer's Director of Clinical Investigation, has just stage-managed a second symposium with a broad interdisciplinary sweep. As if the first of them were not wide-ranging enough, the second was entitled 'Structure in Science and Art', and this time the biologists and philosophers were joined by an architect, a professor of English, two composers, a novelist, three physicists, a concert pianist and an art historian. This heady brew resembled nothing so much as a High Table of High Tables in the Oxbridge tradition, except, of course, that with a battery of microphones and an army of typists waiting to transcribe every slightest remark, conversation was considerably more elevated than standard High Table fare concerning the foibles of students, the price of books and the run-down condition of the college gardens.

This is no place to try to summarise the presentations and discussions, which roamed over perception, the ob-

server in the universe, structuralism in the novel, D'Arcy Thompson's mathematical analysis of form, Breughel's 'Icarus', Schoenberg's piano music and much else besides. But it is appropriate to ask whether at the end of the day any threads, however tenuous, had been laid across the yawning chasm between the sciences and the arts. Does 'structure' offer any common ground—does, for instance, appreciation of the structure of a Mozart concerto help those looking for structure in biological molecules? On the face of it, no.

Those who read the proceedings of this symposium in the hope of seeing new lines of thought emerge, spanning the disciplines, will almost certainly be disappointed. But sensitivity to a wide range of subjects probably does help to enrich work in one's own particular discipline. And occasions such as this symposium do help to heighten that sensitivity of those fortunate participants.

It is a commonplace to say of a conference that the real work was done outside the formal sessions. In this case, however, there is little doubt that this was true; the new contacts, the informal conversations will count for more than what was actually said to the microphones. Mercifully the sponsors seem to appreciate this, and look for no agreed conclusions, no statement to the world out of it all. It is enlightened patronage, of an elitist sort that few dare to pursue these days. □



A week of nuclear decision in the United States and Europe

Columbia University drops plans to operate reactor

LAST week the University of Columbia in New York withdrew a request for a permit from the City of New York to operate a teaching reactor built on the university's Morningside campus in 1969. The university also agreed to withdraw a federal lawsuit, filed jointly with the Department of Justice, challenging the city's jurisdiction over nuclear reactors and radioactive materials.

This move follows a decision by the Faculty of Engineering at Columbia University to accept a request from the president of the university, Dr William J. McGill, not to put the reactor into operation.

Dr McGill had previously resisted moves by members of the local community to prevent the reactor from operating because of health dangers which, some had claimed, would be posed in the case of an accident. However, according to a statement made at a public hearing two weeks ago, Dr McGill said that he had changed his stance mainly in response to public reaction to the Three Mile Island nuclear accident. The university's faculty of engineering has agreed to "acquiesce" to the president's decision. Individually, however, many faculty members disagree with the action, claiming that only increased familiarity with reactor technology will enable operators of the future to avoid the type of mistakes made at Harrisburg.

Students at the university, on the other hand, who have been campaigning against the reactor for many years, claim that the action does not go far enough. They want the reactor, which was constructed in the 1960s but has never been operated due to a succession of legal disputes, to be dismantled, and effort to be transferred to solar energy research.

The teaching reactor is a Triga Mark II reactor, and is licensed to operate up to 250 kilowatts, although university officials say that it would only run at an average of 1 kilowatt. The reactor itself cost about \$500,000, and was financed by the National Science Foundation, with another \$500,000 being spent on the surrounding building. Similar reactors are already in operation in about 60 universities in both the US and overseas.

Earlier this year, after a series of legal battles over who should have the right to licence the reactor Dr McGill announced that the reactor would still go into operation but not until all the legal aspects had been resolved.

Following the Three Mile Island accident, however, and subsequent

student demonstrations on the Columbia campus, Dr McGill announced that he was going to ask the faculty of engineering to reconsider its plans to operate the reactor. The opposition to the university reactor, which he had previously envisaged to be a political problem, "was clearly revealed during the last week in March and the first week of April as a human problem of mounting seriousness," Dr McGill told the public hearing in New York.

"The Triga issue suddenly acquired the potential to tear the campus apart in an explosion of fear and apprehen-

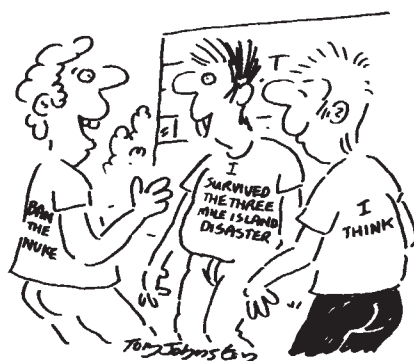
sion. The larger interests of safety and well-being affecting everyone at Columbia University required that I act immediately, and I did so."

Faculty members, while agreeing to go along with the president's decision, are critical both of its implications, and of the way that the decision was taken. "The president's decision was a political one not an educational one; the decision not to operate the reactor is a loss to the country and to the educational community that we cannot afford at the present time," Professor William Havens, director of the university's nuclear science programmes said last week.

The reactor may still be operated in the future, if the general opposition to nuclear power should change, according to Dr Peter J. Likens, dean of the university's school of engineering. "We might seek to reconsider that decision and activate it if the social circumstances mitigate it."

Meanwhile students taking nuclear science and engineering courses at the university may be sent out to the medical research reactor operated by the Brookhaven National Laboratory on Long Island to gain first-hand experience of reactor technology.

David Dickson



"If there's no reactor to learn from, there'll be nobody to build them!"

Punitive damages for Silkwood contamination

IN a decision which could have far-reaching implications for the US nuclear industry, a jury in Oklahoma City last Friday awarded damages of \$10.5 million to the three children of Karen Silkwood, an employee of the Kerr-McGee corporation who, it accepted, had been contaminated by plutonium while working in the company's nuclear fuel processing plant.

Miss Silkwood, a laboratory technician in the plant and an activist in the Old Chemical and Atomic Workers Union, was killed in a car accident in 1974 on the way to a meeting with a newspaper reporter at which, she had said, she would produce evidence that Kerr-McGee had carelessly exposed employees to plutonium. After the accident investigators found traces of plutonium in Miss Silkwood's apartment but no papers were found in the car in which she had been travelling.

After a 10-week trial, the jury found the company negligent in the contamination of Miss Silkwood and her apartment, and rejected the charge that she had contaminated herself with plutonium stolen from the plant in

order to incriminate the company. They awarded Miss Silkwood's estate \$505,000 in actual damages, and \$10 million in punitive damages. During the trial, attorney's for the estate argued that working conditions inside the plant were inherently unsafe, and that workers had not been told that plutonium could cause cancer.

Company officials reported that they had informed the Atomic Energy Commission that they had been unable to account for about 40 lbs of weapons-grade plutonium, and believed that the material was trapped in pipes in the plant; the prosecution attorneys contended that the company's inability to account for the plutonium was further evidence of its negligence. The Kerr-McGee plant at which Miss Silkwood worked has since been closed down.

Agents for Miss Silkwood's estate are bringing a further lawsuit against the Oklahoma City Police Force, the FBI and Kerr-McGee concerning the circumstances of her death and a high OCAW official had called for a special government prosecutor to re-open the case. □

Switzerland increases control of nuclear power

THE referendum on the law governing atomic power in Switzerland last week-end—at which voters approved the government's revision by more than two to one—was hardly the centre of a historic debate. In comparison to the heated debate before the 18 February vote on the nuclear issue—which by a narrow margin upheld the old law rather than one which would have prevented further development of nuclear power—the political temperature has reached an all-time low. All the major parties and organisations either recommended voting for the revision, or refused to take up positions. There has been no propaganda clash. The newspapers are practically void of announcements, and the usual stream of press conferences and meetings is absent.

The main reason for this lack of interest is that the revised law represents a compromise which satisfies no-one. The nuclear lobby is unenthusiastic about the restrictions but reckons "it could have been worse". The only positive aspect it discerns is the clarification of the waste disposal problem (5 April). The anti-nuclear group's general attitude is "better than nothing", although one section voted no, on the grounds that if the revision was rejected, a new revision could only be even more restrictive.

The proposals embodied in the revised law contain enough seeds of discontent to ensure that the peace will probably be only a lull in the storm.

● The granting of skeleton permits for new nuclear power stations, and of construction permits for those already planned—both to be done now by parliament, rather than the cabinet alone—will depend on:

- the demonstration of electricity demand; and
- safe nuclear waste management.

That the law leaves much latitude in the interpretation of these points became clear at a series of hearings in Bern organised by the Swiss Energy Foundation, the main "soft energy" group and leader of the environmentalist opposition to nuclear development. Officials of the various government de-

partments which will be required to put the revised law into practice were interrogated by representatives of both the pro and anti-nuclear groups on their interpretation of the demand and waste management paragraphs, as well as on the proposed procedure for nuclear power stations which already have site but not construction permits.

The transcripts of the hearings¹ indicate that the "demonstration of demand" will become a hard-fought issue in future. How can demand be demonstrated when Switzerland is literally "throwing away" one-third of all the energy it produces because of inefficient systems, poor insulation, lack of incentives for energy saving, etc.? Is not the level of demand really a political question? What reserve capacity is needed to meet short-term abnormal demands or break-downs in functioning units? To decide these questions it is proposed to set up an Energy Commission, but the problem is how to make sure that it is unbiased and uninfluenced by the powerful lobbies.

The "demonstration of safe nuclear waste management" also leaves many questions open. Will it be possible, even with the revised law, to test drill for underground repositories against the will of the local population, without excessive delays? What does safe mean, and how can safety be demonstrated in future waste management when so much of the process takes place outside the country—is what is safe for them, safe for us? Is the question of disposal, particularly of high-level waste, so controversial among scientists that it can hardly be assessed by the present Waste Management Working Group, largely made up of civil servants? How can a waste disposal project be worked out within the next five years (when the first power station falling under the new regulations will be finished) when other countries ahead of Switzerland in R & D envisage 10 more years at least?

Geoff Milnes

¹"Atomgesetzrevision durchleuchtet. Ein Hearing" SES-Report No. 7, Schweiz. Energie-Stiftung, 8001 Zürich.

Gorleben nuclear waste facility scrapped

In West Germany last week the premier of Lower Saxony, Herr Ernst Albrecht, announced that he will not grant approval for the building of a nuclear waste reprocessing and disposal facility at Gorleben. Speaking on television he said the decision was not a technical

one—he believed that the plant would be safe—but he had to take account of the weight of public opinion. However, test drilling in salt domes at Gorleben (see below) will continue.

For a detailed review of the decision, see page 283.



EEC Commissioner opens European nuclear fusion centre

DR GUIDO Brunner, member of the European Commission for Energy, Research, Science and Education, laid the foundation stone for JET, the Joint European Torus, at the Culham Laboratory, UK, last week. During the ceremony, Dr Brunner described JET as a "leading project in the world" in the field of fusion research.

JET's world lead, however, has been

eroded by the delays to the start of the project caused by the two year negotiations in 1975-77 between EEC member states over where it should be sited. The delay has given its nearest rival, the Tokamak Fusion Test Reactor, at Princeton University, US, an eighteen month lead.

The total cost of building JET is estimated at about 200 million Euro-

pean Units of Account (at January 1979 prices). The date for completion is set at the end of 1982 and subsequent annual operating costs are expected to be about £20 million at today's prices. The JET team, which will be drawn from fusion laboratories all over Europe, is expected to be about 320 strong including 120 scientists and engineers. At present

130 people are employed on the project, half of them under Euratom contract, and half of them by the UKAEA which runs Culham.

According to Dr Hans-Otto Wüster, Director of the JET Joint Undertaking and Dr Paul Rebut, leader of the JET design team, there are some worries that JET will not attract enough high calibre fusion physicists to make up the rest of the team. One problem is that during the lengthy debate over where to site the project, almost half the design team returned to their home laboratories. Although many of these have now returned to JET, it is feared that the salaries being offered by Euratom are insufficient to entice French and German scientists to the Oxfordshire countryside.

Initially, experiments on JET will be with a D-D plasma. If these are successful, then the project will move into the "radioactive stage" by 1983-84. This will involve beam-plasma D-T operation resulting in neutron production which will activate the walls of the vessel containing the plasma. Dr Wüster said that this stage of the experimental programme would not commence without further agreement. He also assured that the radioactivity produced would be between ten and a hundred times less than that produced by nuclear fission. As the amount of radioactive material created would be very small, he thought that energy produced from fusion could prove "environmentally advantageous".

The decision over whether or not to enter the 'radioactive stage' is bound to be difficult, according to Dr R. J. Bickerton. There is already one school of thought which would like to proceed to reactor conditions as quickly as possible and another that would rather understand the plasma physics thoroughly first. This difference in opinion is to some extent reflected in the discussions which have already started on the machine to follow JET. Dr Bas Pease, Director of Culham Laboratory, thinks that the next machine should aim at demonstrating a net production of electricity. "I favour the view that we should attempt this after JET, but I can't yet say that I have persuaded a majority of colleagues of that view", he said.

The Euratom countries have already set up a Next European Torus Study Group to discuss the next machine. According to Dr Brunner, however, Europe may not be able to afford to build JET's successor alone. It is therefore cooperating in the INTOR Study Group, under the auspices of the International Atomic Energy Agency, which is looking at the possibility of the USSR, Western Europe, the US and Japan building a worldwide machine.

Judy Redfearn

US foreign research rises to \$1.5 billion

THE vast majority of research carried out by US private corporations in foreign countries is concerned with developing products for local market conditions, rather than with longer term basic or applied research goals, according to a survey conducted by the National Science Foundation.

Research and development carried out abroad by US corporations increased by 41% between 1974 and 1977, to a total of \$1.5 billion, the survey reports. This represents about 7% of the total expenditure on R&D by private companies, which increased by 32% over this period.

The most substantial increase in overseas R&D occurred in the pharmaceutical industry, with many companies conducting trials of new drugs abroad in order to take advantage of more liberal conditions on foreign testing introduced by the Food and Drug Administration in 1975. Thus between 1974 and 1977, the amount of R&D conducted by pharmaceutical companies abroad more than doubled, compared to an increase of only 34% in the funds spent at home over this period (although the report adds that foreign and domestic R&D are now increasing at about the same rate).

The NSF survey says that in companies with world-wide research capabilities, the US laboratory usually takes the lead in overall technological development, with the foreign labora-

tories providing specialised development for particular market conditions. The foreign R&D facilities of these companies therefore conducted primarily development projects, even though basic and applied research accounts for almost one quarter of total domestic R&D spending by US companies.

Interviews carried out with the managers of 18 large private corporations revealed only one case in which a company reported any expenditure on basic research conducted outside the US. Two other companies reported that their foreign research facilities carried out some applied research work.

Looking to the future, the NSF study says that an expected increase in overseas sales by US corporations is likely to cause such companies to open new foreign R&D facilities or to expand existing operations, with increased sales, rather than other factors such as lower research and development costs, providing the main motivation for such developments.

The report also notes that several R&D directors outside the pharmaceutical industry said that increasing regulation by bodies such as the Occupational Safety and Health Administration and the Environmental Protection Agency in the US would tend to cause companies to move R&D resources to other countries where operations would not be affected as much. □

University of Houston expels professor

THE University of Houston in Texas is refusing to renew the contract of Professor Archer J. P. Martin, joint winner of the Nobel prize for chemistry in 1952 with R. L. M. Synge for his work on chromatography, on the grounds of "inadequate productivity".

Professor Martin, who is 69, was appointed to the Welch chair of chemistry in 1974, and until last year held a joint appointment at the University of Sussex, where he was carrying out research on protein separation sponsored by the Medical Research Council.

The University of Houston requires that after a faculty member reaches the age of 64, his or her tenure must be renewed annually. Last year the university told Dr Martin that the Department of Chemistry did not consider he had published enough scientific papers, and that his appointment would not be renewed.

Professor Martin told *Nature* last week that when he took the post, he had not been aware that there would be little money for research assistants or experimental facilities and consequently that he spent most of his time in Houston planning experiments to be carried out in Sussex. □

He accepts that he has published very few scientific papers over the past five years from his work at Houston, but claims that he has no desire to rush into print unnecessarily (his total scientific output is about 70 scientific papers). According to Professor Martin, the university did not take into account papers produced from the work at Sussex in considering whether his contract should be renewed.

A faculty committee, to which Professor Martin protested at his dismissal, came to the conclusion that the terms of his appointment had not been initially made adequately clear, and recommended that he be kept on the staff. However, two weeks ago Professor Robert H. Walker, Dean of the College of Natural Sciences and Mathematics, informed Professor Martin that the university had no power to keep him on unless the Department of Chemistry changed its mind. According to Dr. Walker, the university does not set any specific target on the number of papers which a research worker is expected to produce each year but, he says that there is nevertheless "a certain expectation of productivity". □

Congress goes easy on science—so far

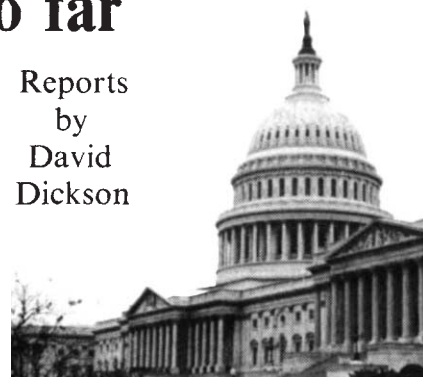
A KEY subcommittee of the US House of Representatives last week recommended that \$19 million be cut from the budget request for the National Science Foundation which had been submitted by President Carter. Although this represents a reduction of almost 2% in the \$1,006 million request submitted. It is considerably less than the \$44 million reduction which the same subcommittee wanted made in the NSF's budget last year.

The subcommittee's recommendations, which have to be accepted by the full committee of Appropriations before being debated on the floor of the House, would delete \$10 million from the agency's basic research programmes out of a requested total of \$793.3 million, \$3 million out of the \$62.4 million requested for the applied science and research applications programmes, and \$3 million out of programme management.

The subcommittee made no mention of the ceiling which Congress placed on salaries of NSF grant-holders last year, leading some to speculate that the ceiling might be lifted during conference with the Senate. However, neither did it add any extra money for research on earthquake hazards, for which the House last month authorised spending to be increased from \$18.3 million to \$22.8 million.

NSF officials were relieved that the cuts were not greater (a move to make an additional \$12 million cut is said to have been headed off in the subcommittee meeting). However, the real tests will come in the middle of next month when the NSF request is debated both on the floor of the House—where the Appropriations Bill has already been cut back by \$14 million—and in the Senate Appropriations Subcommittee chaired by Senator William Proxmire. □

Reports
by
David
Dickson



Funds denied for nuclear waste

THE House Armed Services Committee has voted to cut off all funds for an experimental project to store nuclear waste in salt caverns near Carlsbad, New Mexico.

The project, known as a waste isolation pilot plant (WIPP) has been under development by the Department of Energy for some time. The site was chosen in 1976, and if permission to build the plant is granted, the site could be ready to receive both transuranic wastes from the defence programme and commercial waste from nuclear reactors within a few years.

The scheme has generated intense controversy in New Mexico. Supporters say the WIPP facility would bring new jobs and extra income to the area. Opponents claim it would restrict access to other valuable resources, such as potash, and also point to doubts over the suitability of salt as a disposal medium.

The House Armed Services Committee voted against further funding for the WIPP facility not on environmental or scientific grounds, but because it felt that a plant initially intended for defence wastes should not also be used for the disposal of commercial wastes. Meanwhile, the Senate Armed Services Committee has agreed to let funding for the project stand in the Department of Energy's budget request, but on the understanding that no commercial spent fuel should be stored at the WIPP site.

The outcome will depend on what happens in floor debates and during the conference between the House and the Senate. Another factor will be the President's announcement soon on federal waste disposal, following a report on nuclear waste management prepared last year by an interagency review group.

Meanwhile, the Senate and the New York State Assembly announced last week a curb on the amount of nuclear waste that can be stored in the state's West Valley facility, which has been closed since 1972. □

Senate committee kills fast breeder

A NEW political confrontation over the future of the liquid metal fast breeder reactor project at Clinch River in Tennessee seems certain after the Senate Energy and Natural Resources Committee last week accepted a proposal from Senator Dale Bumpers of Arkansas to kill the \$2.6 billion project. The House Science and Technology Committee has already voted to continue.

President Carter has consistently been opposed to the plan, initially on the grounds that the use of plutonium in fast breeders increases the chances of nuclear proliferation. More recently, the President has claimed that the Clinch River design was obsolete after new advances in technology, and that with a slowing down in growth of energy demand, the need for fast breeders is not so urgent.

The General Accounting Office, however, which carries out studies of particular issues for the Congress, disagrees with the President. In a report published earlier this month, the GAO stated that the Clinch River project would be a "logical and prudent step".

In pledging its support for the project, the House Committee added \$184 million to the authorisation request for the Department of Energy so that the project could proceed. Senator Bumpers' proposal, which has been accepted by the Senate Energy Committee, is to terminate the breeder project by 1 October, but to authorise a design study for a larger breeder test plant. "Clinch River is dead and the sooner we admit it, the more the American people will applaud us for doing it," Senator Bumpers said. □

Technical aid restored to UN bodies

THE US Senate voted last week to repeal a decision, taken in the heat of the closing hours of the 95th Congress last autumn, which effectively cut off all US contributions to bodies in the United Nations system.

The earlier decision took the form of an amendment, proposed by Senator Jesse Helms of North Carolina, which specified that none of the funds which the US contributes to bodies such as the World Health Organisation and the Food and Agricultural Organisation could be used for the purpose of "technical assistance".

The UN bodies subsequently told the US State Department that it could not

accept contributions under those conditions. Last week, however, in passing the Foreign Relations Authorisations Act for 1980, the Senate in effect repealed the Helms Amendment, following a similar move in the House of Representatives.

The Senate also defeated, by 54 votes to 35, a further amendment from Senator Helms which would have revived the issue by refusing to include money for technical assistance in the mandatory contributions which the US pays to the UN bodies. Senator Helms said it was a matter of whether the Senate wished to impose an 'International Tax' on Americans. □

Pesticide resistance on the increase, says UNEP

RESISTANCE to pesticides has been spreading so rapidly among pests that no manufacturer cared to offer a new pesticide to the World Health Organisation for safety testing in 1978. It now takes millions of dollars to test the safety of new chemicals and often within months pests start developing resistance to it. WHO recently reported that it is now cutting down its field staff involved in safety testing of pesticides.

Reflecting these events, this year's State of the Environment Report to be released by the United Nations Environment Programme on the occasion of the World Environment Day (5 June) will focus upon the increasing dangers posed by pesticide resistance. The report makes quite frightening reading.

It points out that in 1965, the Food and Agricultural Organisation listed 182 resistant strains of arthropod pests; in 1968, it listed 228 resistant species; and now its latest survey of 1977 lists 364 species.

There are now 223 agricultural pests resistant to nine of the major groups of pesticides. Many of these, says the UNEP report, "are major pests of major crops, such as cotton bollworm, the boll weevil, and the leafworm of cotton, the rice stem borer and the brown plant hopper, the Colorado beetle of potatoes, spider mites of fruit and glasshouse crops, and cutworms and weevils of cereals".

Up to 1970, very few resistant plant pathogens had been observed. But with the introduction of new systemic fungicides, the resistance problem has crept in, and now more than 35 species of plant pathogens have been reported as resistant.

Rodents, which destroy crops, are also becoming resistant to rodenticides. Latest FAO figures show that seven species of rodents, including two important and widespread species, *Rattus rattus*, and *Rattus norvegicus*, have developed resistant strains.

If there is any ray of hope it seems to be with weeds, which have not yet shown any sign of developing genetical resistance similar to that occurring amongst insects—even though herbicides now account for some 50% of all pesticides applied. For long-lived perennial and vegetatively reproducing weeds, says the report, "the potential for development of genetical resistance seems low bearing in mind that experience with insects and other classes has demonstrated that in the field many generations must pass before such resistance reaches notice-

able levels".

The most worrying situation is in the field of public health. WHO figures cited in the UNEP report show that there are now 121 resistant strains of insects important to public health campaigns compared to 102 in 1968. In 1969, 15 species of anopheline mosquitoes were resistant to DDT and some 37 to dieldrin. In 1976, some 43 species were known to be resistant to dieldrin, 24 to DDT, five to organophosphates like malathion, and two to carbamates.

Resistance to insecticides is now found amongst anopheline mosquitoes in 62 countries out of 107 where there is malaria. In several parts of the world there has been a massive resurgence of the disease, with some countries showing a 30-40 fold increase in the number of malaria cases since 1968-70.

Switching over from DDT to other insecticides like malathion has not proved easy. Most of the substitutes of DDT are both much more toxic and much more costly. India's malaria control programme alone consumes nearly 60% of the Indian government's health budget.

Amongst culicine mosquitoes, vectors of such diseases as yellow fever, filariasis and dengue, resistance has increased from 19 species in 1968 to 41 species in 1975. Other important vectors such as houseflies, black flies and fleas are also becoming resistant. The house fly, says the UNEP report, seems to be the insect "with the greatest ability to develop resistance to insecticides over the widest geographical area". A total of 121 resistant strains of housefly were reported in 1975.

Research on pest control agents with novel modes of action like chemosterilants, hormones and growth inhibitors, and biological agents like bacteria, viruses and fungi, has been hailed in the belief that pests would be less likely to develop resistance to them. The UNEP report however points out that this hope is ill-founded.

Resistance has already appeared to hormone-based pesticides where little resistance had been expected. "New compounds such as growth regulators and microbial pesticides have not been in use long enough or on a wide enough scale to show perceptible resistance, but here again as with chemosterilants, it has been possible in the laboratory to develop resistance by artificial selection."

The flour beetle, *Tribolium*, has not



only developed multiple resistance to conventional pesticides but also acquired significant cross-resistance to the growth inhibitor, methoprene. It has also been shown that houseflies can become resistant both to the spores of *Bacillus thuringiensis* and to its toxin. *B. thuringiensis* is expected to become an important biological control agent.

What then is the solution to pest control? UNEP points out that the classical alternative approach of changing the pesticide when faced with the problem of resistance is a practical solution only in the short term. Alternative pesticides need to be developed but in the long term, the situation is such that it requires the development of alternative strategies.

Instead of total reliance on just one type of control agent like chemical pesticides, UNEP favours the concept of 'integrated pest control.' This would include elements of chemical pest control, environmental control of breeding habitats, genetic control techniques, biological control, behavioural control using sex pheromones and related compounds, and breeding of resistance in crops and animals.

Integrated pest control strategies will have to be specific to a pest in a specific environment. This will require considerable research. An FAO/UNEP panel of experts on integrated pest control has over the last few years developed a Global Programme for Integrated Pest Control in relation to various priority crops like cotton, rice, sorghum, maize, millet, roots and tubers and grain legumes, and is now extending this to vegetable crops.

Studies conducted in South America using integrated pest control techniques on cotton—the highest consumer of insecticides in agriculture—shows that the quantity of chemicals needed can be reduced by a third, thus cutting down costs and health risks and increasing productivity. But while this system of integrated pest management has been considerably developed, both in theory and in practice, as regards agricultural pest control, the UNEP report claims that extensive research programmes are still needed to apply it to public health vector control.

The switch from chemicals to the new packages of integrated practices is therefore likely to take a considerable time.

Anil Agarwal

Ecology groups combat Chinese irrigation plan

CHINESE plans to divert water from the Yangtze to irrigate northern China have run into cautiously expressed but clear opposition from Chinese environmentalists. A recent meeting of the Chinese Society for Water Conservancy, officially described as an "academic discussion" on the plan, concluded that in the past, "our improper water control plans violated objective laws". Any plans to divert the Yangtze, they stressed, must be considered not from a purely engineering point of view, but should also take into account possible ecological changes produced by the scheme.

As far as the engineers are concerned, plans still remain flexible. A national forum held in Tianjin at the beginning of April discussed three possible routes: the "Western route" from the mountainous upper reaches of the Yangtze via "snaking tunnels" to north-west China; the "middle route", via a projected canal which would cross the Hwang-Ho near Zhengzhou; and the "eastern route" which would roughly follow that of the Grand Canal built by the Emperor Yang Di in about 600 AD.

All three routes, the forum decided, were practicable, although each plan still required some improvement. Construction of the western route, it was concluded, lay beyond China's capability at the moment. The eastern route, first announced in August 1978, although feasible, attracted considerably less support than the middle route, which appeared to be the choice of the Yangtze Basin Planning Commission. A spokesman for the commission told the forum that this route would involve the construction of a 1,000 km canal from the Banjiangkou reservoir on the Han river (a major tributary of the Yangtze), as far as Peking, via the foothills of the Funiu range and the Nanyang basin in southern Henan, approaching Peking on a route parallel to the Peking-Canton railway. When completed, he said, the scheme would irrigate 4,666,000 ha of farmland, bringing an average of 10,000 million m³ water annually to the Hai river basin, thus equalling the annual flow of the entire Hai system.

However, it might seem strange to divert water from the Yangtze across the Hwang-Ho. Why not simply irrigate north-west China with water diverted from the Hwang-Ho? The reason is that the mean flow rate of the Yangtze



is 34,000 m³ sec⁻¹, considerably greater than the 1,500 m³ sec⁻¹ flow rate achieved on average by the Hwang-Ho.

The spokesman admitted, however, that there was one major disadvantage to this plan. The annual output of the Danjiangkou hydroelectric station would have to be reduced by anything from 700 million to 1,700 million kWh annually. This, however, he claimed, could be compensated by the Gezhou power station now under construction further westward.

At least one water conservancy specialist, a delegate from Qinghua University, showed some support for the plan. He noted that large areas of rock fissures and sandy structures beneath Peking city could be used as an emergency water store, while some 15,000 sq km of 'abandoned river beds' north of the Hwang-Ho could be used to store 39,000 m³. In general, however, the conservationists are somewhat cautious. The "academic discussion" of the Conservancy Society pointed out a number of factors which demand further consideration. Would the diversion, they asked, cause secondary salinisation and alkalinisation of the soil? Further, what would happen in drought years? In 1978, droughts in the middle and lower Yangtze basin and the consumption of large quantities of water for irrigation led to a back-flow of sea water into the Yangtze estuary, so that, for a time, the population of Shanghai was obliged to drink brackish water, while at Chongming island, which supplies agricultural and market-garden produce to Shanghai, cultivation was seriously affected. Any proposed diversion of the Yangtze, they stress, "must conform to the law of nature and the economic law".

Vera Rich

Hungarian scientists want better links with government

HUNGARY'S Academy of Sciences has adopted a new constitution which calls for greater accountability from government departments and industry.

Although in July 1969 the Central Committee of the Hungarian Communist Party affirmed, in a startling change of direction, the need for complete freedom in scientific research and an atmosphere of free and uninhibited discussion among scientists, economic demands, both national and within the Comecon framework, have led to a policy of closer integration between research and production. This the Hungarian scientists seemed perfectly prepared to accept. Last year's General Assembly of the Academy stressed the need for greater selectivity in research subjects, taking into account the current "social needs".

Nevertheless, this linkage does not seem to be operating as smoothly as hoped. There are considerable delays (running into years and sometimes decades) in the introduction of Hungarian patents and inventions in practice. This is not only due to bureaucratic delays; last summer, the president of the National Patent Office, Emil Tasnadi, told the prestigious political and literary journal *Elet es Prodalom* that the fault lay largely with the scientists. There are some 35,000 scientists in Hungary, he said, but their sole aim is to achieve academic degrees: the titles of Candidate (PhD) and Doctor bring with them, respectively, a life-long stipend of 500 and 1,000 forints per month. Since these degrees are earned through the publishing of articles and books, he said, and not by inventions, only six researchers in every thousand actually have a patent to their credit.

While not actually answering Tasnadi directly, Dr Janos Szentagothai, the president of the Academy, took the opportunity of this year's General Assembly to state the scientists' case. Links between research and production have come closer in recent years, he noted approvingly; nevertheless, the situation is by no means satisfactory. The various departments of the Academy duly make the proper recommendations to the production sector—but receive little or no feedback. Frequently, he said, the scientists do not know if the recommendations have even been discussed.

The new constitution of the Academy, adopted by the General Assembly on 11 May, and made retroactive to last April, demands that this situation should be rectified. □

news in brief

Harvard makes mathematics mandatory: In a far reaching revision of its undergraduate curriculum Harvard University will demand mathematics proficiency from its entire student body. All Harvard entrants will be required to show proficiency within their first year in three areas: applications of the function concept to the real world; probability and statistics; and in the use of time-shared computers. The mathematics requirement will have further effects in raising the level of instruction of the new science requirements for non-science students. All students will be required to take a one semester course in a physical science ("predictive and deductive analysis and quantitative treatment of data") and a one semester course in a biological science ("descriptive treatment of complex historical or evolutionary systems"). Harvard's move, because of its great prestige in the US, is expected to have widespread implications for the whole of US higher education.

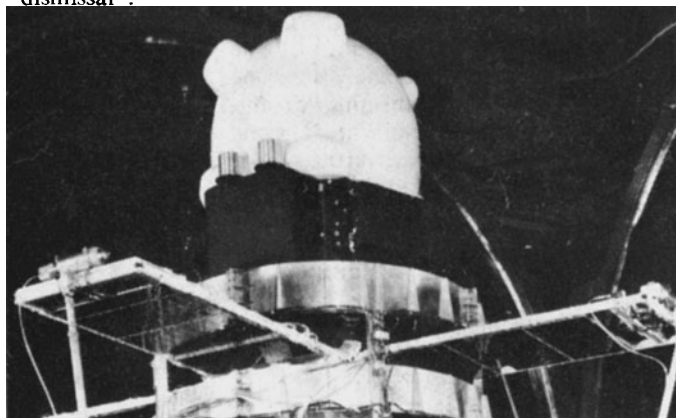
Spanish dockers block uranium import: The Spanish dockers union has boycotted the vessel *Covadonga* carrying nuclear fuel from the US for a power station under construction at Leimoniz in the Basque country. The boycott applies to every port in Spain and the vessel was diverted to Bordeaux. The Spanish unions appealed to unions throughout Europe for solidarity and French dockyard workers refused to handle the cargo. The ship is now reportedly bound for Cadiz in Spain. The Leimoniz power station is strongly opposed within Spain; it has been the subject of armed attacks by Basque separatists who have the support of the Left opposition and the trade union movement.

New study challenges efficacy of nuclear energy: A new study claims that nuclear power is "technically incapable of providing a timely and significant substitute for oil." A 50% replacement of oil by nuclear power by the year 2000 would require ordering one large power station every 3.5 days. The study, by Amory Lovins of Friends of the Earth, argues in addition that the energy supply problem is "90%," one of heat and portable liquid fuels. Lovins says that if every oil powered station in the OECD in 1975 had been replaced overnight by nuclear stations the fraction of OECD oil which is imported would have only been reduced from 65% to 60%. And at the same time there would be a greatly increased dependency on imported capital and uranium. The maximum role for nuclear power is in providing electricity which is only 10% of all primary energy. Lovins also reviews 19 studies from eight countries and estimates that conservation measures can hold steady or reduce energy needs while maintaining economic growth. Two British studies indicate that growth can triple while total energy demands could be reduced by one half.

"Is Nuclear Power Necessary?" by Amory Lovins. Friends of the Earth, 9 Poland Street, London W1V 3DG.

Pillinger to appeal case: David Pillinger, a biochemist made redundant at the Christie Hospital in Manchester (25 January) will take his case to an appeals tribunal. An industrial tribunal ruled against Pillinger last month finding that he had not been unfairly dismissed from his permanent post as Senior Grade Scientist at the Christie because of a lack of funding from the Medical Research Council. The appeal must be based on a point of law with no further consideration of the facts of the case. The question to be raised will "involve the whole three cornered relationship between employer, employee and granting agencies" says Pillinger. In Pillinger's case, a third party has been per-

mitted to exercise the right to hire and fire without giving reasons for its action. According to Pillinger's solicitor the case is "fundamental to the whole concept of unfair dismissal".



UK6 due for launch this week: The UK's sixth scientific satellite (above) is due for launch this week aboard a NASA Scout rocket. It is the last of the UK/Ariel series and carries three high energy astrophysics experiments: a cosmic ray detector for studying ultra-heavy cosmic rays; an x-ray telescope system for studying x-ray sources in the range 0.1–2.0 keV; and experiments for observing variable x-ray sources with high time resolution in the range 1.2–50 keV. UK6 will be the last purely British scientific satellite for some time at least although the UK is still negotiating with NASA over the development of a multi-mission refurbishable satellite.

EEC takes steps to meet energy crisis: After a delay caused by UK objections to EEC coal buying policy, the Council of Energy Ministers has approved European Community Commission research proposals covering energy saving, geothermal energy, solar energy and coal gasification. The Council has allotted 55 MEUA (£36m) over four years for energy saving projects and 95 MEUA (£63.3m) for research into alternate energy sources. In the UK, the EEC has provided 21 MEUA (£14m) for nine demonstration projects in energy saving. The applications selected under the scheme include three heat recovery projects (in steel making, plaster board manufacture, and in a swimming pool recreation complex) five heat saving manufacturing devices and a monitoring system of energy consumption.

In the meantime, the Union of European Community Industries (UNECI) has issued a report calling for a massive expenditure of 373 billion UA for investment in energy production and an additional 350 BUA for energy prospecting and for research and development. The UNECI report estimates that 650 new coal mines, six natural gas fields, and oil production equivalent to seven Niagras and 600 nuclear power stations will be needed to meet a projected doubling of energy requirements by 2000 AD.

Skylab falls soon: The US National Aeronautics and Space Administration announced last week that it was expecting the 85-ton Skylab space station to fall to Earth between 26 June and 9 July, with 2 July as the most probable date. According to the agency, most of the space station, which was launched in 1973 for carrying out research into activities in space, will burn up as it enters the atmosphere. However between 400 and 500 pieces are now expected to reach the surface of the Earth, scattered along a path 4,000 miles long and 100 miles wide.

Gorleben: winning the battle, losing the war?

Helmut Hirsch, coordinator of scientific opposition to West Germany's nuclear reprocessing plans, gives his view of the decision last week not to proceed—for the time being

THOSE of us closely involved in the debate over whether or not to build a nuclear waste disposal centre at Gorleben have, it now seems, been barking up the wrong tree. The Federal Government stated two years ago that one of the conditions for further nuclear expansion in West Germany was what it called the "technical safety and feasibility" of nuclear processing and reprocessing. It was on this basis that the Reactor Safety Commission and the Commission for Radiological Protection expressed their opinion that the Gorleben centre was feasible. It was also on this basis that 20 international scientists, working in the Gorleben International Review, produced a 2,200 page report arguing that it was not. The matter was discussed at the controversial hearings in Hanover which ended on 3 April.

Now, it seems, we were all wrong. Because on 16 May Minister-President Albrecht of Lower Saxony revealed to the astonished German public that he was not worried about Gorleben's "technical safety and feasibility". His chief concern, he said, was its political feasibility. He turned the project down because of political, not technical, opposition.

Albrecht was well aware of the massive opposition to the centre; during the hearings, more than 100,000 people demonstrated in Hanover. He made quite clear in his speech on 16 May that he considered the roots of this opposition were wholly irrational but he decided he would not ram nuclear power down the throats of the ill-informed populace. Or at least he would not do so as long as he was the only one responsible.

Albrecht (who belongs to the CDU) is clearly very irritated about the position adopted by Lower Saxony's Social Democrats. Their leader, Ravens, came out with the conclusion that the Gorleben centre was technically not feasible. Ravens even met Chancellor Schmidt, an outspoken supporter of the Gorleben plan, but Schmidt did not pull Ravens into line. Thus, Albrecht, when announcing his decision, made it clear that he would not take it upon himself to push ahead with reprocessing plans which originated in the Federal Government's (and hence the SPD's) energy strategy but which were opposed by the SPD

in Lower Saxony, who could then profit from their opposition by gaining anti-nuclear voters.

Albrecht also announced the next step: *intermediate* storage of spent fuel elements, probably for up to 40 years, while research into final disposal is to be intensified, covering both post-reprocessing wastes and unprocessed spent fuel elements. However, it is the Gorleben saltdome—and only the Gorleben saltdome—that is to be explored for its suitability for final disposal. If the results are positive, disposal of radioactive wastes will take place there; if not, other sites will then be considered. Clearly the people of Gorleben will have to postpone their victory celebrations.

Local people fear—perhaps with good reason—that this means Gorleben is still the first and only choice for solving West Germany's nuclear waste problems, particularly if pressure mounts for an official decision on a suitable site so that the country's nuclear capacity can expand as fast as possible. They are less than confident that the exploratory drillings at



Minister-President Ernst Albrecht: turned down Gorleben plan for political reasons

Gorleben will be analysed in an unbiased manner, and fear that the saltdome will be proved suitable simply because the nuclear lobby needs it badly.

This concern is shared by Dr Friedrich Mauthe, geologist at Hanover University and one of the participants of the hearings. He says, "So far, nobody has even bothered to define clear criteria stating which drilling results are necessary for a positive judgement. I wouldn't feel too happy if the criteria are tailored after the drillings have been completed".

The procedure envisaged by Albrecht goes right against the conclusions of the Gorleben International Review, whose report emphasised that a broad investigation at several sites would be necessary before any site was singled out. The review expressed doubts about the Gorleben saltdome as well as the suitability of salt as a medium in general. The GIR proposed

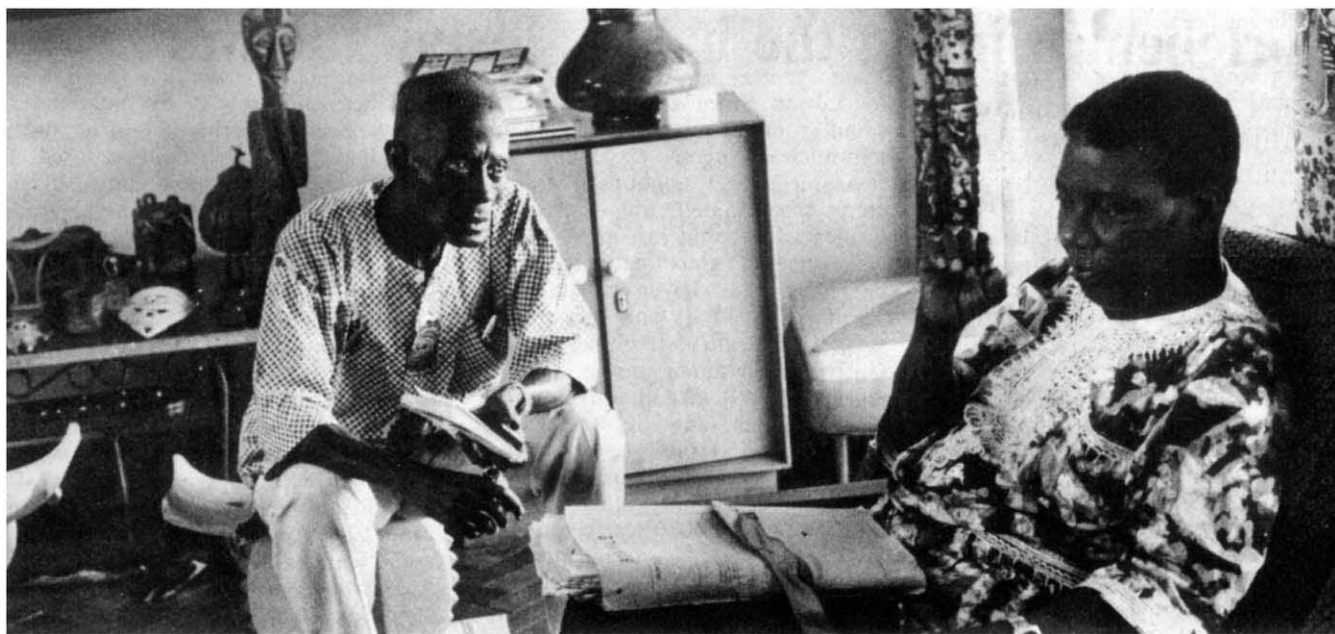
detailed and timely investigations of other geologic formations. But at the hearings, Albrecht flatly refused to discuss the Gorleben saltdome.

Albrecht has avoided the question of where to site the intermediate storage facilities. This is another worry for the people of the Landkreis Lüchow-Dannenberg, to which Gorleben belongs. They might yet have to put up with storage ponds. The siting of these at Gorleben would further suggest that the nuclear industry still wants to proceed with the original proposal, with some modifications, and use step-by-step tactics to avoid major conflicts. Considering the determination with which the Federal Government has pushed reprocessing up to now, these suspicions may not be completely unfounded.

Given the uncertainty and political manoeuvring, who can blame the people of Lüchow-Dannenberg if they take things into their own hands? On 10 May, 600 local inhabitants walked into a closed meeting of local politicians discussing the Gorleben issue at Hitzacker, frustrated with the attitude of their representatives, many of whom still have not made up their minds. One farmer shouted: "We do not want the plant, and we do not like it better if it comes step by step!"

Activists collected more than 20,000 signatures in just over two weeks—in a district with 48,000 inhabitants, including children—and are still working to complete their list. The official farmers' union has come out with a clear "no" against nuclear facilities. The resistance is strengthened by the fact that test drilling has been going on at the site more or less continuously for the past two months. The latest news was that drillings had revealed a layer of peat 50–70 m below the surface. This is not unusual over a salt dome, but it indicates that the ground is not suitable for large concrete buildings.

The people of Lüchow-Dannenberg were not surprised by Albrecht's decision. Marianne Fritzen, one of the leaders of the local opposition says: "Albrecht is very cautious, but it is clear to me that he is still in favour of the reprocessing plant." However, many opponents of the project feel that at least a battle has been won. Time has been gained which can be used for a national debate on the issue which really ought to have been paramount: the desirability of reprocessing, and of further use of nuclear energy in general, which has been tacitly assumed by German politicians right from the start. □



"I decided to learn from them": Dr Oku Ampofo (right), director of the Centre for Scientific Research into Plant Medicine, and one of his herbalists

When the scientist meets the medicine men

Joseph Hanlon reports from Africa on the increasing respectability of 'primitive' herbal healing

RESEARCH into medicinal plants is now a high priority in Ghana, and it typifies the issues in science there because it is essentially local and yet, at the same time, tied to the developed world.

Herbal medicine researchers use sophisticated analytical chemistry techniques, collaborate with laboratories all over the world and publish in prestige western journals. Yet they rely on the knowledge of traditional healers to solve problems of tropical medicine which have been ignored by a medical science dominated by the developed countries. Herbal medicine conjures up images of witch doctors and 'primitive' cures. In fact, western drugs came almost entirely from plant and fungus extracts until the 1930s. And many drugs, particularly contraceptive pills, still do.

Plant medicine is still the norm in most of the world, and is not being swept aside by synthetic drugs. One reason is that medicinal plants work. Analysis shows they contain many compounds already familiar to pharmaceutical chemists and do have the antibiotic and other properties claimed for them. Indeed, some work for ailments which cannot be effectively treated by modern "scientific" drugs. Herpes zoster (shingles) is an example.

This point has not been lost on multinational pharmaceutical companies. Faced with spiralling research costs, they are paying more attention to traditional medicines and Third World research. Which leads to the sharpest

contradiction for Ghanaian scientists. They are dependent on high technology only available in European and US labs, and collaboration with those labs is crucial. Yet that means the drug companies are likely to get their hands on the results first. Some scientists fear that new drugs discovered in Ghana and other developing countries will be patented by the multinationals and then sold back to developing countries at high prices. The Organisation of African Unity (OAU) has gone so far as to urge secrecy in herbal medicine research to prevent just this.

Publicity about Chinese traditional medicine systems—both acupuncture and herbal—gave traditional healing international respectability, and plant medicine is now being promoted by the World Health Organisation. Before WHO gave its blessing, however, Ghanaian scientists had already turned to this field.

The Faculty of Pharmacy at the University of Science and Technology (UST) in Kumasi has for several years worked primarily on plant medicines. A number of scientists at the University of Ghana and Korle Bu Hospital are also working in this field. Ghana's Centre for Scientific Research into Plant Medicine, founded four years ago, is one of four centres with official OAU backing.

The centre's founder and director is Dr Oku Ampofo. He qualified at Edinburgh in 1939 and returned to his home town, Mampong-Akwapim (now the

site of the centre). "The war was on, and there were no medicines to be had. There were some old people in the district who were treating patients very effectively with herbal medicines. I saw that some of these things worked. Take the case of a woman bleeding after childbirth. In those days, we were far from any hospital to deal with such an emergency. But they had wonderful herbs for post-partum haemorrhage. So I decided to learn from them." Over the years, he studied with a variety of herbalists and compiled a list of more than 300 remedies.

The Ghana government is spending more than £1 million building the new centre, which will have a staff of 20 scientists. Although the building is several years from completion, two scientists have already been hired and sent abroad for further training.

The centre will also contain a clinic both to treat local people and as a research base. The clinic is already operating in temporary buildings. Three doctors and three herbalists work together, treating patients and keeping careful records. One pilot study has already found several herbs that are as effective as the anti-diabetic drug chlorpropamide. The centre has its own arboretum to grow useful local plants and it has just been granted a part of a forest reserve in the Accra Plain where it can grow savannah plants from northern Ghana.

The first research step is always to identify as many active compounds as possible. If one is found with a structure akin to a known pharmacologically active substance, then it explains

how the medicine works, and also serves as proof to doubting Western trained doctors who continue to resist plant medicine. Indeed, some of the chemists I spoke to considered their most important role to be convincing doctors that science has an explanation for these herbs. Pharmaceutical chemists are happiest working with compounds close to those they know and understand.

One of Dr Ampofo's plants now being tested at UST is *Cryptolepis sanguinolenta*, which is sold in the local markets, soaked in water and drunk as an anti-malaria drug. It contains a compound very similar to quinoline.

The same plant is also used by herbalists to reduce fever and to cure urinary infections. Microbial tests show that it is a broad spectrum antibiotic, though none of the compounds isolated so far is a known antibiotic. This is an increasingly common occurrence and has led scientists to catalogue the plants in their traditional form, so they can be sold in shops and used by doctors and herbalists who are not familiar with them. Many of the plants are quite common. Using them directly, instead of waiting for compounds to be analysed, synthesised, and converted to tablet form, makes them widely available much more quickly. The Pharmaceutical Society of Ghana now has a policy that where the scientific basis of a remedy can be shown, and where toxicity tests have been conducted, pharmacists should prepare dosages of the crude plant, rather than attempting extracts. Skin ointments, worm expellers and purgatives are now on the market.

Testing for safety and side effects

But this, too, has its problems. Plants vary widely according to soil and the time of year (and sometimes even the time of day) when they are picked. Some plants can be highly toxic at certain times of the year and not at others. The herbalists know their local plants well, but this doesn't help those without specialist knowledge. Storage also remains a problem. And the method of preparation is often not precise: "Put a little bit into water"—but how much? Dr Ampofo, through his clinical tests, and the UST scientists, by attempting to find the active compounds, are trying to standardise doses.

Traditional medicines, too, have their side effects and some safety testing seems called for. Dr Ampofo notes that "experienced herbalists will warn you that the plant may have a side effect and if it does, to take another preparation to counter it". Also, "most tropical plants contain poisonous alkaloids. In some cases, if one plant is

used for a treatment, then another must be taken as well to eliminate the toxic effect. *Elaeophorbia drupifera* is useful for guinea worm. The herbalist taps the juice and mixes it with palm nut oil, which renders it non-toxic. Otherwise it is poisonous—you purge yourself to death. I know of one herbalist who has an excellent plant for rheumatism, coroinanthe pacchyceras. But it only works in an alcoholic medium—pound it and put dessert-spoonfuls of powder in 20 ounces of gin. Then take one teaspoonful after each meal. If you boil it up instead, it will put you to sleep for two days."

Traditional healers have been ridiculed in the West—and by western-trained Ghanaians—because of the continued failure to find "scientific" justification for their claims. The Ghanaians argue that western scientists are so confident of their own methods that they are unwilling to listen to anyone from a developing country, despite the hundreds of years of careful study of plant medicine by the herbalists. Many Ghanaians consider Western science too reductionist, constantly trying to find a single extract that can be synthesised and put into tablet form. "I don't think the western world knows how fully to analyse a plant yet. There are certain enzymes we know little about and cannot isolate, but which seem to do good work. In any case, isolation of a single compound cannot be the be-all-and-end-all—it may be a synergistic action in the plant that is doing the healing," Ampofo argues.

He criticises scientists who "follow a routine method of analysing all plants, without knowing anything about what they do clinically. They take a long time and then say it's no good. But if you know that plant A stops bleeding, you straight away look for coagulant effects. You don't just go through to get a long list of formulae."

Another reason to listen to herbalists is to find out the method of preparation. "I found a plant which is extremely good for infectious hepatitis —*Bombas boboperzenze*. You cut the bark to pieces, put it in cold water, and put it in the sun for half a day before drinking. I gave it to a professor and he worked on it for a couple of months and then said he couldn't make head or tail of it. I found out that he had boiled it. If the herbalist tells you to do a preparation in a certain way, it doesn't matter if you are a PhD, you must still follow it exactly."

This new scientific approach has already led to promising research on plants for hypertension a problem in village Africa as much as urban England), guinea worm, skin infections, and many other conditions. By looking in their own gardens, Ghanaian scientists have been able to do research

that could not be done elsewhere. They have staked out an area of scientific research and produced world class results which are being published in the top medical and pharmacological journals.

But the going is not easy. Modern analytical chemistry requires sophisticated equipment that Ghana cannot afford. Advanced drug research today is beyond the means of Ghanaian universities. For example, Dr Ivan Addae-Mensahe, a Ghana University chemist, has isolated an amide alkaloid, wisanine, from *Piper guineense*, the Ashanti pepper. The roots and seeds of this plant are used for a wide variety of conditions, including venereal diseases, mumps, and intestinal disorders. It is also used as a sedative. One alkaloid of the plant, nitidine, has been shown to lower blood pressure, so there is considerable interest in the plant as an anti-hypertensive.

Can Ghana keep the drug companies at bay?

But Addae-Mensahe could only do part of the work. "With my own equipment, I could isolate the compound and tell if it was pure. I could tell the class of compound, its functional groups, and that the structure was close to something I knew. But I needed mass spectrometry and nuclear magnetic resonance—which I didn't have—to tell the detailed structure." His papers on wisanine cite laboratories in Freiburg, London and Cambridge for analytical work. Other work has been done in Australia and Chicago. Much of the work was done as favours by other scientists—his adviser at Cambridge, a scientist he met in Nigeria, and even a contact made by his brother who studied in Germany. The antibacterial properties of wisanine were discovered in Germany; its effects on hypertension are being assessed in the US.

Similarly, discovering that *Cryptolepis sanguinolenta* contained a quinoline-like compound was beyond the capability of UST. They had to rely on friends and their own students in universities in Pittsburgh, Bradford and Upsala. The next step is to conduct tests on monkeys, but Ghana has no facilities. As the multinational pharmaceutical companies are now concerned about the rise of strains of malaria resistant to present drugs, they may take over the work.

Where does this leave the Ghanaian scientists? They are concerned that scientific knowledge is far from universal when patents and profits are concerned. Are they, like the cousins on the farms and in the mines, merely supplying raw materials for the multinationals in the developed world? □

Another cancer scare . . . or is it hypochondria?

WHERE will the search for environmental carcinogens end? A recent article (Commoner, Vithayathil, Dolora, Nair, Madyastha, Cuca, *Science* **201**, 913; 1978) implicates extract of cooked hamburgers; another, (Bruyninckx, Mason, Morse, *Nature* **274**, 606; 1978) oxygen at physiological concentrations. Both were found to be mutagens in the Ames test. Mutagenicity in this case is usually, though not always, associated with carcinogenicity in mammals.

Commoner *et al.* found that ground beef cooked in a home hamburger cooking appliance contained a substance that induced mutations (reversion from histidine dependence to histidine independence) in some strains of *Salmonella typhimurium*. In the *Science* article the authors are properly cautious: "If . . . these mutagens—once purified and tested on laboratory animals—are found to be carcinogens, their apparent concentration in some foods may represent an appreciable risk to certain populations." But the media were less restrained: these cautious claims were blown up in the United States into the Great Hamburger Scare of Fall 1978. Professor Commoner was widely quoted in the press and on TV, and MacDonald hamburgers' stock prospects were re-examined by alert Wall Street analysts.

The Bruyninckx *et al.* findings are even more startling. It has been known for 25 years that hyperbaric oxygen is a mutagen—but mammals are generally not exposed to hyperbaric oxygen. Bruyninckx found that exposure of certain mutants of *S. typhimurium* to 5% O₂-5% CO₂-90% N₂ induced as much as a fiftyfold increase in reversion to histidine independence compared to controls kept under anaerobic conditions. In speculating on the significance of their findings, Bruyninckx *et al.* say, "According to Fridovich oxygen toxicity is normally held in check by a balance among rates of formation and destruction of reactive forms of oxygen. This may mean that oxygen mutagenicity is improbable, but not impossible, in normal aerobic mammalian cells; but higher rates of formation of reactive forms of oxygen or lower rates of their destruction . . . could lead to significant rates of mutagenesis along with the molecular pathologies arising from mutation." Thus Bruyninckx *et al.* do not quite say that oxygen, in the form of the O₂⁻ radical, may be implicated in carcinogenesis—but others, notably J. Totter, former Director of the Division of Biology and Medicine of the US Atomic Energy Commission, have suggested just this.

Alvin M. Weinberg, director of the Institute for Energy Analysis, turns his attention to the carcinogenicity of oxygen



If oxygen is dangerous, we'll have to stop breathing . . .

These two findings suggest to me that our entire approach to cleansing our environment of carcinogens may be bankrupted by further investigation. Today's environmentalism assumes that environmental agents that do harm, particularly those that cause cancer, are important causes of cancer compared to the natural environment, and also are removable. But these two doctrines have already been shaken by such findings as the presence of nitrosamines in experimental animals fed a normal diet; or for that matter, the existence of the radiation background, not to say of sunlight itself. Professor Commoner's hamburgers are almost unavoidable (though his directions for cooking mutagenic hamburgers may reduce even this exposure). But I would defy even the most ingenious environmental regulatory agency to legislate acceptable levels of oxygen!

Obviously we must get a better idea of how much cancer is attributable to agents that are in principle removable. The oft-quoted assertion that as much as 80% of cancer is caused by environmental agents that are, at least by implication, avoidable, rests on evidence that can hardly be considered compelling, a point recently stressed by Peto (*Nature* **277**, 428; 1979). To be sure, cancer maps show large fluctuations in incidence of specific cancers in different locations; but the fluctuations are much smaller if all cancers in one location are compared with all cancers in another location.

So far regulatory agencies have not raised seriously the question of how much a known carcinogen can add to the unavoidable risk of cancer. If, for example, it turned out that an all-pervasive environmental agent such as oxygen is importantly implicated in cancer, then we may be attacking the one-tenth of the iceberg that shows (the avoidable carcinogens), but ignoring the nine-tenths that is submerged (the unavoidable carcinogens). I offer this speculation to bring home the great difficulties our regulatory agencies face in trying to legislate acceptable levels of exposure.

I should think that before they outlaw MacDonald's hamburgers, or for that matter, before scientists call for a total ban on this or that carcinogen, we await further clarification of the role of all-pervasive agents such as oxygen in the etiology of cancer. We need more scientific understanding much more than we need additional regulation that is based on imperfect and fragmentary evidence.

Where does the scientist's responsibility lie—in publicising the possibility that a commonly used substance might be a carcinogen (Chicken Little), or in withholding publication until he can really assess the risk, say, compared to other carcinogens (Dr Pangloss)? Chicken Little adds to the public's growing environmental hypochondria; Dr Pangloss conceivably might fail to alert the public to a potential danger. Until now Chicken Little has been in fashion, but I hope the pendulum will swing toward Dr Pangloss. The public ought to require of scientific Chicken Littles the same norms of conduct that science itself has imposed: cautious, provable scientific assertions, and a minimum of appeals to the unrefereed public press.

If such an attitude leads to more Panglossism so be it: people will never stop eating hamburgers, let alone reduce their oxygen uptake, no matter what our scientific Chicken Littles and our regulators urge. □

news and views

Decaying charm

from D. J. Miller

THE charmed particles are proving a great deal harder to study than the strange particles were. Both the charm quantum number and the strangeness number are conserved by the strong and electromagnetic interactions. This means that charmed or strange particles must undergo weak decays whose rate is determined by two factors; the weak-interaction coupling constant G_F and the density of allowed final states—the phase-space available. By a happy chance the characteristic lifetimes of strange-particle decays are around 10^{-13} s, so for a particle travelling close to the velocity of light the decay-lengths are around a centimetre or more. This is ideal for bubble-chamber work, where the resolution is approximately half a millimetre, and a wealth of precise data now exists on the lifetime and decay schemes of all the basic strange states. Even the elusive Ω^- hyperons are now being studied by the hundred.

But although charmed-particle decay processes are also governed by G_F , the charmed particles are much heavier than strange particles. This leads to a much greater density of allowed final states for each decay-mode and the predicted lifetime drops to the region of 10^{-13} s, giving decay-lengths of a few hundred micrometres; too short for any normal bubble-chamber to observe. In fact, it is quite difficult even to produce charmed particles in a bubble chamber. Most of our knowledge of them comes from the e^+e^- colliding-beam machines where D , D^* and other charmed states form a significant part of the total rate, once the charm threshold is passed at about 3.8 GeV

(see *News and Views* 269, 286; 1977). Yet such machines have even worse spatial resolution than a bubble chamber, so the presence of the charmed particles is deduced from observation of their decay products with no hope of measuring the decay-lengths of the parent particles. Nuclear emulsions are, so far, the only detectors which offer a chance to see charmed particle tracks. A lone candidate was reported 3 years ago by an international collaboration, using a stack exposed at Fermilab near Chicago (Burhop *et al. Phys. Rev. Lett.* 65B, 299; 1976). Now a continuation of the same collaboration has reported three new events (Angelini *et al. Phys. Rev. Lett.* 80B, 428; 1979; and Ankara, Brussels, CERN+University College Dublin, University College London, Open University, Pisa, Rome, Turin collaboration preprint, to be published) seen in a stack which was placed just in front of the BEBC hydrogen bubble chamber in the CERN SPS neutrino beam. A few percent of charged current neutrino events are known to give charmed particles in the final state. Without the information from the bubble chamber, and its associated muon detector to establish the changed current, it would take hundreds of man-years to scan a large emulsion stack for a useful number of these events. By spotting likely events in the bubble chamber pictures and extrapolating the tracks back to the emulsion it has been possible to find 101 neutrino interactions in emulsion in less than 2 years. The bubble chamber also gives an opportunity to measure the momenta of tracks leaving the

emulsion and even in some cases to identify the kind of particle which produced a particular track. But the emulsion gives the vital information—charmed tracks are indeed a few hundred micrometres long. Unfortunately, for three of the four events reported so far, the information on the momenta and identities of the secondary particles from the charmed decay has not been good enough to allow the masses and momenta of the charmed particles to be established. There is, however, one event in which a strong case can be made that the decaying particle is the charmed baryon Λ_c^+ and, if that is assumed, the momentum can be estimated with some precision. The two figures show tracks from this event. In the emulsion (Fig. 1) a single lightly ionised track travels from the neutrino interaction-star on the left of the picture and splits up into three light tracks after 354 μm . These particles all give corresponding tracks in the bubble-chamber picture (Fig. 2), marked a, b and c in the figure. In particular, track c makes an elastic scatter from a hydrogen nucleus in the bubble chamber (see arrow on figure). It has been possible to fit this interaction and identify track c with a high confidence-level, as a proton track. Track a does not match quite as well as the other two with its counterpart in the emulsion—it may have scattered slightly in the steel wall of the bubble chamber.

Ionisation measurements in the emulsion, together with the curvature of the tracks in the bubble chamber, indicate that track a is most probably due to a K^- and track b to a π^+ .



Fig. 1

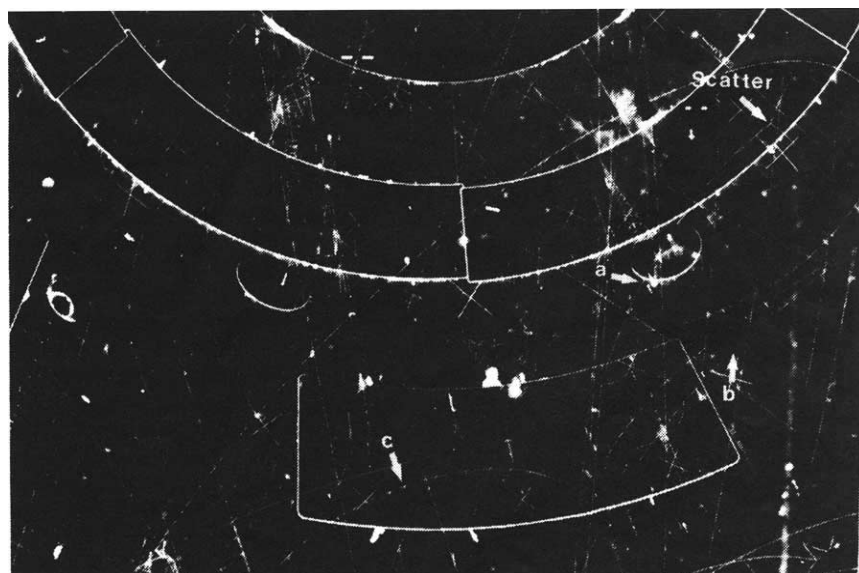


Fig. 2

Taking the best information on all three tracks the event is consistent with production of a hyperon of mass $2.3 \text{ GeV}/c^2$ and momentum $3.74 \text{ GeV}/c$. It decays to $K^-\pi^+p$ after living $7.2 \times 10^{-13} \text{ s}$. The other three events have similar decay lengths so their decay times must be in the same region.

Because the charmed quark is thought to be much heavier than the everyday u and d quarks or the strange s quark, when a charmed particle decays the process should be simpler than in strange particle decays. To predict the rate for a particular strange decay requires a detailed discussion of the dynamics of two or three-body final states, with special enhancements and even *ad hoc* selection rules. Charm decays, in the current weak interaction theory, should have about the same rate whether the charmed quark is in a charmed baryon or in a charmed meson. The energy released is much bigger than the variations in mass between baryons and mesons, so the quark can decay at its own pace and the final state is rearranged over a comparatively long period afterwards. (This is the same sort of 'parton' argument that is used to explain the deep inelastic interactions of electrons or neutrinos with quarks, quark-pair production in e^+e^- annihilation or large transverse-momentum jet production in proton-proton collisions). It is thought likely, in view of the number of $K\pi\pi$ events seen in searches for charm in bubble chamber neutrino events (Baltay *et al.* Columbia-Brookhaven collaboration; *Proceedings of the Oxford Neutrino Conference 1979*, page 198, published by the Rutherford Laboratory) that most of the charmed particles produced by

neutrino interactions are D mesons, not Λ_c hyperons. Some of the other emulsion events are consistent with many-body D decays, so it is encouraging for theorists that the apparent survival times of all events are similar to that of the Λ_c event.

This event is also encouraging on a more fundamental level. As soon as charm was taken seriously it was possible to predict the whole $SU(4)$ spectrum of charmed mesons and baryons. In the past 5 years many dynamical effects have been explained by the existence of charm, but few members of the charmed spectrum have been firmly established. The Λ_c has been reported before (see for example Cnops *et al. Phys. Rev. Lett.* **42**, 197; 1979 and references therein) but each piece of evidence was slender. A well reconstructed Λ_c track is a considerable advance. $\square$

Bacterial defences against penicillins

from a Correspondent

WE cannot always take a detached view about the outcome of conflicts between one form of life and another. We seek to kill pathogenic bacteria with antibacterial agents—but this onslaught may be resisted by the bacteria, which may produce (or contain) an enzyme that destroys the drug. Penicillins and cephalosporins are among the most effective antibacterial agents and they owe their activity to the presence of the four-membered β -lactam ring. Enzymes that catalyse the hydrolysis of the β -lactam ring—and consequently inactivate the drugs—are called β -lactamases. The increasingly wide occurrence of bacterial β -lactamases has become a source of concern to clini-

cians and a challenge to chemists and biologists. Hence inactivators of β -lactamases are not only tools for enzymologists, they are also potential chemotherapeutic agents. It was against this background that a workshop* was held recently at the University of Newcastle upon Tyne to discuss the biochemistry of bacterial β -lactamases. There have been significant advances in our understanding of β -lactamase action since the first workshop was held, 4 years ago (see *News and Views* **255**, 526; 1975). Moreover, then we had no effective inactivators of these enzymes; now we have several.

Information about structure, although often laboriously acquired, is particularly significant and enduring. All our knowledge of amino acid sequences in β -lactamases has come from the laboratory of R.P. Ambler (University of Edinburgh) recently—and dramatically—complemented by the work on the nucleotide sequence of a plasmid that encodes a β -lactamase (J. G. Sutcliffe, Harvard University). It is noteworthy that the β -lactamases from *Staphylococcus aureus*, *Bacillus licheniformis*, *Bacillus cereus* (β -lactamase I) and *Escherichia coli* have amino acid sequences that are sufficiently similar to show that they must be classed as homologous proteins. We may thus expect that one mechanism will apply to all these enzymes. By way of contrast, Ambler reported that β -lactamase II from *B. cereus* had so far shown no sequence similarity to the other β -lactamases, and he concluded that this is a strong candidate for an analogous (as opposed to a homologous) enzyme. Moreover, β -lactamase II is exceptional in requiring $Zn(II)$ for activity. S. G. Waley (University of Oxford) (reporting on work carried out in collaboration with H. A. O. Hill and E. P. Abraham) described spectroscopic evidence that the ligands to the essential metal atom were three histidine residues and the enzyme's sole thiol group.

The intermediates in the pathway of synthesis and export of the extracellular β -lactamase of *B. licheniformis* now include species of molecular weight 35,000, 33,000 and 31,000 (J. Lampen, Rutgers University, New Jersey). Structural characterisation of these interesting intermediates is, however, not yet complete.

Information about the three-dimensional structure of β -lactamases is eagerly awaited; J. R. Knox (University of Connecticut) described his progress, both on the β -lactamase from *E. coli*, and on the transpeptidase from *Streptomyces* R 61; which of the structures being studied in several labora-

*Held on 27–29 April and organised by Professor R. H. Pain and Dr R. Virden (University of Newcastle-upon-Tyne). The workshop was supported by Glaxo Research Ltd.

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tories will be solved first remains to be seen.

Turning from structure to mechanism, one of the highlights of the meeting was J. R. Knowles's (Harvard University) presentation of the first good evidence for an acyl-enzyme intermediate in the enzymic reaction. The hydrolysis of a 7- α -methoxycephem (cefoxitin) by the RTEM β -lactamase from *E. coli* proceeds slowly enough for an intermediate to be isolated at 0 °C; the low molecular weight moiety is covalently bound. When the reaction in solution was monitored by Fourier-transform infrared spectroscopy, the intermediate showed a band at 1753 cm^{-1} . This is consistent with the intermediate's being an ester, and this, in turn, implicates a serine or threonine residue in the enzyme's action. The importance of serine in β -lactamase I follows from the work of P. G. Sammes (University of Leeds) and Waley who have found that the amino acid residue labelled by an inactivator is serine 44 (serine 70 in Ambler's method of numbering). This is a conserved residue in the sequences referred to above. These results, and those of Knowles, taken together suggest that β -lactamases may now be regarded as 'serine enzymes'. The inactivator used by Sammes and Waley (which was independently discovered by R. S. Pratt, Wesleyan University, Connecticut) was 6- β -bromopenicillanic acid, and the labelled enzyme is thought to be a dihydrothiazine. A. F. Coulson (University of Edinburgh) used the sulphone of 6- α -chloropenicillanic acid to inactivate the β -lactamase from *Staphylococcus aureus*: the site labelled is close to (or identical with) that referred to above. The chemistry of the inactivation by these reagents entails acylation followed by fragmentation, according to the views of those using these inactivators, but the structures postulated have yet to be firmly established.

The detailed studies by R. H. Pain and R. Virden (University of Newcastle-upon-Tyne) on the conformation and activity of the β -lactamase from *S. aureus* have led to a three-domain model, with the active site at the interface of two domains. This protein is one of the few for which a partially unfolded form has been well characterised.

Methods of obtaining information about the practical importance of β -lactamases in protecting bacteria against the antibiotics were discussed by G. W. Ross (Glaxo Research, Greenford) and by M. H. Richmond (University of Bristol). Mutants of certain strains of *E. coli* have been obtained lacking the barrier to inflow of antibiotic, and these are helping to throw light on the least-understood

aspects of β -lactamases. These concern the relative positions (in Gram-negative bacteria) of the β -lactamase and the target that the antibiotic interacts with.

As usual, much remains to be done, but significant advances have at last been made in the understanding of these efficient but unwelcome enzymes.

High energy jets

from M. G. Albrow

In the past few years high energy physicists have progressed beyond the study of the interactions of the strongly interacting particles, the hadrons, to the study of the interactions of their constituents. All known and postulated observable 'elementary particles' fall into three classes (see the review article by Mulvey *Nature* **278**, 403; 1979). These are the leptons (electron, muon, tau and neutrinos), supposedly fundamental particles with half a unit of intrinsic angular momentum (spin) which have no strong interaction coupling, the hadrons (proton, neutron, pions, kaons and so on) which interact strongly, and the field bosons (photon, graviton, intermediate vector bosons) which are the quanta of the force fields acting between the leptons and hadrons. It has become universally accepted that the hadrons are not truly elementary particles but are composite objects. They are probably composed of two types of constituent—quarks, which are very similar to the leptons except that they have a non-zero strong charge, and gluons, which are the field bosons or quanta of the strong force. Thus all matter consists of spin $\frac{1}{2}$ particles either with no strong charge (leptons) or with a strong charge (quarks) interacting by exchanging field quanta (bosons).

And yet despite extensive attempts to kick a quark or gluon out of a proton and observe it as an isolated entity, none has succeeded. One can set up a collision experiment that should produce a quark, even to the extent of knowing in which direction it should be produced and with what momentum. In that direction one finds not a single particle but a set of several hadrons moving in roughly the same direction, with a total momentum equal to the momentum the quark should have had. It is just as if the quark produced immediately breaks up into more familiar particles. Such a set of hadrons is called a 'jet', with a jet axis defined as the direction of the vector sum of their momenta. Each member particle has a momentum component parallel to the jet axis and a component transverse to that axis. An observed charac-

teristic of jets is that the average parallel component grows linearly with the total jet energy, while the transverse component remains small, a few hundred MeV/c. Consequently as the total jet energy increases, the angles between the member particles decrease and the jets become more collimated. In the highest energy collisions the jets produced become sufficiently distinct that the multi-jet configuration of the resulting particles appears. Even though in a very high energy collision dozens of particles may be created, it is now postulated that they are all members of a small number (typically 2–4) of these jets. That is to say that a small number of axes can be found such that no hadron has a large transverse momentum component to all the axes. If each jet is taken as signifying the direction of a quark or gluon the description of the collision process takes on a remarkable simplicity.

How do these jets appear, in the reference frame in which the total vector momentum of all the particles is zero (the centre-of-mass frame)? In Fig. 1a an electron and a positron collide; they annihilate and all of their energy is converted into hadrons. Typically two collinear jets of hadrons are observed, the axis making an angle θ with the collision axis. The angular distribution has a form $(1+\cos^2\theta)$, which is exactly that expected if just two spin $\frac{1}{2}$ particles, namely quarks, were produced and then disintegrated into hadrons. Collisions of this type were first seen at SPEAR in Stanford, California in 1975 (Hanson *et al. Phys. Rev. Lett.* **35**, 1609; 1975) and become much more striking at the higher energy now available at DESY in Hamburg (Berger *et al. Phys. Lett.* **78B**, 176; 1978).

In Fig. 1b an electron–proton collision is shown, with the electron emerging intact after suffering a deflection and loss of energy. Again the created hadrons seem to group themselves into a pair of jets which are however not collinear. One of the jets recoils against the deflected electron and its internal structure appears similar to that of the jets produced in e^+e^- annihilation, suggesting that a quark has been kicked out of the proton and has subsequently disintegrated in the same way. The rest of the hadrons form a second jet pointing along the direction of the incident proton—presumably arising from the remaining proton constituents after one quark has been removed.

A stage further in complexity is the proton–proton collision seen in Fig. 1c. Whenever particles with large transverse momentum relative to the collision axis are observed, they occur in pairs of jets approximately balancing each other in momentum. The natural

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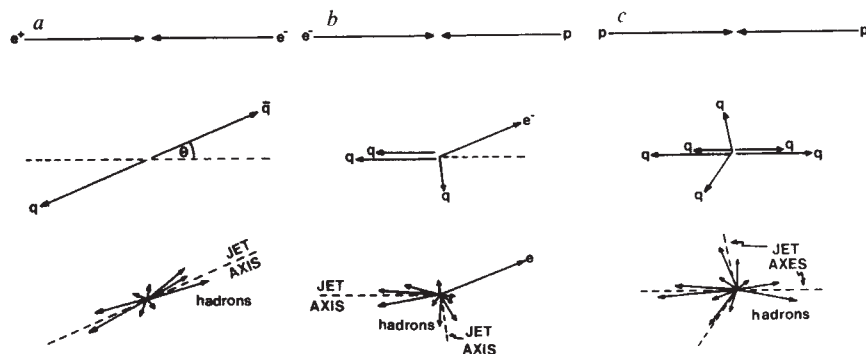


Fig. 1 *a-c*; Three classes of high energy particle collisions producing jets of hadrons. The initial state, the inferred primary products and the final state hadrons are shown as momentum vectors.

interpretation is that a pair of constituents (one from each proton) has scattered through a large angle.

From these three types of collision process studied at three quite different types of machine (e^+e^- colliders at SPEAR and DESY, large accelerators at FNAL and the CERN SPS, and the CERN proton Intersecting Storage Rings (ISR) a unified programme of research has now emerged. The first process (Fig. 1*a*) is the ideal way to study the mechanism by which quarks evolve into hadrons, the second (1*b*) can measure the distribution of quarks inside a proton and the third (1*c*) can study the quark-quark scattering process. This threefold attack on the study of the strong interaction was the subject of a Jet Symposium held last year at the Neils Bohr Institute in Copenhagen (*Physica Scripta* **19**, (2) 1979).

Quite how the emerging quarks turn themselves into hadrons is still something of a mystery. Our present understanding of the strong forces between quarks is based on quantum chromodynamics theory (QCD). In this theory the quark-quark force increases as their separation increases, unlike electromagnetic forces where the reverse is true. It may then take an infinite amount of energy to separate a pair of quarks to macroscopic distances. As quarks separate, the energy contained in the increasingly strong field builds up until it is sufficient to 'polarise the vacuum', resulting in a newly created quark-antiquark pair. This process repeats itself until most of their kinetic energy is spent, and the created quarks and antiquarks arrange themselves into hadrons. Unfortunately it is not yet possible to calculate the details of this complicated process. It is still an open question whether free isolated quarks can be produced in sufficiently high energy collisions—it is conjectured, but not yet proved, that they are permanently confined. In that case we may now be exploring the last rung of the ladder down from atoms through nuclei and elementary particles to their most fundamental constituents. □

Arabic science at Aleppo

from Subir K. Banerjee

FOR historians of Arabic science an important meeting* took place in Aleppo, recently. The President of this young university Ahmad Y. Hassan, himself a noted historian of technology, has single-handedly provided the impetus for the creation of the world's first major centre devoted to a comprehensive study of all the Arabic sciences, the Institute for the History of Arabic Science at Aleppo University. The institute is also responsible for the publication of the *Journal for the History of Arabic Science*, the first journal of its kind. The term Arabic Science is used in preference to Islamic Science, perhaps to emphasise the idea that we are dealing here with the history of science (and technology) written in the Arabic language which was the language of rational inquiry in the Occident during the period AD 800–1400.

Why should anyone study Arabic science? Before we in the modern West mumble something about its impact on the European Renaissance it is worth recalling what E. S. Kennedy, one of the doyens of studies in Arabic mathematics and astronomy has said, "There need be no laboring of the conceited notion that the providential function of the Arabs was to preserve Greek science against the time when Western Man should awake from his slumbers of the Dark Ages". Taking this view one step further I would like to say that while intrinsically laudable, it is an extremely parochial and ethnocentric view to emphasise in the study of Arabic science only the role of transmission to the Latin West at the cost of minimising the grandeur and

breadth of Arabic science as science *per se*. It is a sobering thought that perhaps only 10% of the total Arabic scientific oeuvre is known to have been translated into Hebrew and Latin. The growth, maturity and decline of Arabic science should be studied primarily as the flowering of human mind during a certain era and for the lessons it can offer to all mankind.

The symposium was successful in driving home this broader viewpoint of Arabic science. One interesting contribution in the sessions on the 'Transmission of Arabic science to the Latin West' was Marie Thérèse d'Alverny's discovery of the important influence of Arabic cosmology in the 12th century *Dialogus* of Peter Alphonsi. A highlight was A. I. Sabra's talk in the general session on the place of science and medicine in medieval Islamic civilisation. Over the past few years in private conversations and in short articles Sabra has indicated that he is struggling with the problem of decline of Arabic science and at the symposium he was able to state clearly his new approach to the discussion of this problem: he is concentrating on the careers of the individual scientists of this age, their training, flourishing and activity later in life. In the topical sessions I attended it was most exciting to hear of G. Saliba's identification of the Damascene astronomer al-Urdi (13th century) as the original author of the planetary model which constituted an alternative to the Ptolemaic model and that 'Urdi's model appears to have been adopted by the more well-known Quth ad-Din ash-Shirazi. Other interesting new information selected at random is that the Arabs had a magnetic compass for scientific use as early as 13th century and that a 14th century Aleppan astronomer, as-Sarraj, had invented an exceedingly complex universal astrolabe which, additionally, had the equivalent of a slide rule on its back for complex calculations in spherical astronomy, the trisection of an arbitrary angle and the nature of the lunar surface. □

Timing time

from P. C. W. Davies

IT has been predicted for 60 years that time runs faster in space than on Earth. Now the prediction has been verified by a direct experiment in which a high-precision clock—an atomic hydrogen maser—was flown into space on board a spacecraft. In a recent issue of *General Relativity and Gravitation*, two Harvard astrophysicists, R. F. C. Vessot and M. W. Levine, report the preliminary results of the experiment,

*The Second International Symposium for the History of Arabic Science was held on 5–12 April at Aleppo, Syria.

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which took place over the Eastern United States in June 1976.

The effect being verified is caused by the action of gravity on time, which forces clocks (and all natural activity) to run more slowly in the Earth's gravitational field. At high altitudes where gravity is lower clocks speed up. The phenomenon follows directly from the assumption that energy has weight, through a simple argument. When a body is lifted it acquires some extra energy by virtue of the work expended to elevate it. If the energy that drives a clock (for example, elastic energy of a spring or vibrational energy of a balance wheel) also has weight, then additional work must be performed to lift it: crudely speaking, a ticking clock is heavier than an inert one. The extra work expended appears as enhanced internal energy which thereby induces the clock to run more energetically, that is, faster.

The time distortion effect is a completely general one and is really a property of time itself, not merely a mechanical shortcoming of clocks. It is a central prediction of Einstein's general theory of relativity because weighing energy amounts to checking the so-called equivalence principle—that gravity is locally indistinguishable from an acceleration. The effect is minute in the case of the Earth, but in certain astronomical objects such as black holes, it can become enormous.

In 1959 a modest precursor of the Vessot-Levine experiment was performed at Harvard University by R. V. Pound and G. A. Rebka. Very finely tuned gamma rays produced using the Mössbauer effect were shot vertically up a tower 22½ metres high and their frequency at the top compared with that at the base. The tiny shift of only a few parts in 10^{15} could be detected by compensating for it with the Doppler effect, which shifts the frequency of radiation from moving sources. The spacecraft experiment improves on this by probing much greater changes in the Earth's gravity than from the bottom to the top of a tower. The payload was projected 10,000 km into space atop a Scout D rocket launched from Wallops Island, Virginia. At this altitude the time distortion is as much as five parts in 10^{10} , well within the detection capabilities of modern atomic hydrogen masers.

The principle of the experiment was simply to compare the beats of the maser in space with two standard Earthbound masers. In practice there are great complexities involved because other more prosaic effects, such as ionospheric disturbances and Doppler shifting due to the spacecraft motion,

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More news on SS 433

from A. C. Fabian

THE moving emission lines of SS 433 were one of the most exciting astronomical discoveries last year (see *News and Views* 277, 267; 1979). Since the object became visible in the night sky this year many telescopes have been following its behaviour. At the Spring meeting of the American Physical Society in Washington, DC, Bruce Margon (University of California, Los Angeles) reported that the enormous variations in line position are due to the Doppler effect. He and colleagues at the University of California and elsewhere have been prominent in observing and unravelling this bizarre spectrum, and find that all emission lines visible in their spectrographs are split into three components. Those either side of the component at rest wavelength move in the opposite sense and are displaced by an equivalent velocity which varies up to $+52,000 \text{ km s}^{-1}$ and $-30,000 \text{ km s}^{-1}$. The asymmetry is likely to be due to the transverse Doppler effect in emitting matter that is travelling (probably in two diametrically opposite directions) at about $80,000 \text{ km s}^{-1}$. The change in velocity represents an acceleration $\sim 1g$ over several weeks. The overall variation appears to recur on a period of 160 ± 3 days (or some multiple of that). The radial velocity curve passes through a cycle of ~ 104 d which is repeating this year within 2% (including some small but significant variations). The remaining part of the curve should be visible this summer.

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Margon emphasised that this is the first time definite evidence has been found for large scale matter moving towards us at relativistic speeds—even the Paschen lines can be seen in the visible at maximum blueshift. Simply interpreting the radial velocity curve as due to a binary system leads to the picture of two masses of $2 \times 10^6 M_{\odot}$ orbiting each other just beyond their respective Schwarzschild radii. Perhaps more plausible would be to consider that the emitting matter lies in two jets which are waving around with a period of 160 days. Radio astronomers find evidence for jets in radio galaxies, and relativistic matter squirting out of a galactic nucleus seems to be necessary in order to explain the superluminal expansion in many of these sources. SS 433 may represent a much scaled down galactic version of one of these nuclei. I currently favour a precessing neutron star as the underlying source of energy—Margon has noted that the inferred matter velocity in SS 433 is close to the velocity of escape from such an object. (As an aside it may be noted that SS 433 could be used by an interstellar civilisation to thrust them up to relativistic speeds!)

SS 433 would be visible in binoculars if its position in the galactic plane did not cause significant dimming of its light. Nevertheless, it is within the grasp of many telescopes and must represent one of the most spectacular objects visible. Special relativity can now be demonstrated at significant fractions of the velocity of light on something more than subatomic particles. The emitting mass in SS 433 exceeds $10^{14} g$.

have first to be taken into account. One vital requirement was to obtain accurate information about the spacecraft trajectory, because the time distortion effect is a function of altitude. The accuracy aimed for is about $\pm 100 \text{ m}$ in position and $\pm 6 \text{ cm s}^{-1}$ in velocity near apogee, though the authors admit that as yet substantially larger uncertainties exist. Further data reduction will be undertaken to improve the understanding of the trajectory, especially at low altitudes. On the basis of current data, the clock rate distortion predicted by the general theory of relativity is verified to two parts in 10^4 .

The principle of equivalence can be checked in other ways, such as the Dicke-Eötvös experiment, which uses a type of balance at a fixed location on the Earth, and is found to be correct

to one part in 10^{12} . If the principle is assumed correct, then the Vessot-Levine experiment can be used instead to check that the velocity of light is independent of direction by comparing the one-way and two-way light travel time from the ground to the spacecraft. In fact, the authors comment that there are a whole range of possibilities opened up by the use of high precision clocks aboard spacecraft.

About 70 years ago Einstein and others developed the theory of relativity by appealing to so-called 'Gedanken' experiments in which observers undergo hypothetical experiences involving gravitational fields and different states of motion, while equipped with measuring rods and clocks. In those days spaceflight and super-accurate technology were merely a dream. Now, less than a century later,

some of these fantasy experiments can actually be performed, and the measuring rods and clocks, albeit in technically unrecognisable form, have become a reality. Vessot and Levine point out other experiments which are feasible with current technology using clocks in space. One of these is the measurement of the Lense-Thirring frame-dragging effect, which is a sort of vortex in space in the vicinity of a rotating massive body. Determination of the magnitude of the effect due to the Sun by orbiting clocks near the solar surface in a drag-free satellite, would enable an accurate measurement to be made of the total angular momentum of the Sun, a quantity which is crucial to our understanding of stellar structure. Another possibility is the detection of very low frequency gravity waves which, when washing through the Solar System, cause long-period fluctuations in clock rates. There is no doubt that as technology improves, more and more of the exceedingly minute, though highly significant distortions of space and time predicted by Einstein's theory will come within the scope of direct experimental verification. □

Micro-ecology

from N. MacDonald

A RECENT review in *Nature* (Bloom 279, 21; 1979) of immunological aspects of parasitism, discusses the methods adopted by parasites to survive within host cells. A full issue of the *Proceedings of the Royal Society* (B204, 1979—arising from a meeting held last year) has recently been devoted to wider aspects of the cell as a habitat for other cells, covering mutualistic interactions as well as parasitism. The evolutionary implications of early symbionts (cells inhabiting cells) which may have become integrated into the structure of more complex cells (present-day mitochondria and chloroplasts for example), have been widely discussed, and are reviewed by F. J. R. Taylor and by J. M. Whatley *et al.* (These and all subsequent references are to papers in the previous reference). The general topic of the micro-ecology of contemporary symbionts, their mechanisms of adaptation to the intracellular environment, and the regulation of their populations by that environment, is probably less widely known.

Various kinds of association of host cell and symbiont can be arranged in order of increasingly close integration. Most symbionts are segregated by en-

closure in a membrane-lined vacuole. Those that are not can typically not be grown outside the host cell. These are presumably candidates for a process in which they lose their separate identity and merge with the host cell, at any rate in cases where the association is mutually advantageous.

This potential loss of identity is not the only way in which the cell as an environment is vastly different from the physical environments of large-scale ecology. Regulation of the population of the symbiont is necessary lest it grows to the extent of bursting the host cell, as well as for restraining the competition of the symbiont for resources needed by the host. In all cases of stable association in which the symbiont is capable of separate existence, the maximum intracellular growth rate of the symbiont population is less than the maximum extracellular growth rate (D. C. Smith). Some of the regulatory mechanisms (as discussed for example by L. Muscatine and R. R. Pool for intracellular algae) are reminiscent of those encountered in animal ecology, at least in so far as one regards the environment of a particular species as including its predators and competitors. The host cell may expel the symbiont, consume it or starve it by competing for a resource. One radically different mechanism, for which there is now some evidence, is the triggering of mitosis in the symbiont by mitosis of the host.

J. W. Moulder discusses the cell as an extreme environment, explicitly examining the partial analogy with physical extreme environments, such as hot springs or salt lakes. Such environments are characterised by low diversity of the species that inhabit them; frequently there is a single dominant species. This species is likely not only to have adapted to the presence of the primary non-biological limiting factor, such as temperature or salinity, but to have become dependent on it. The limiting factors of a host cell are of a more varied and active nature. The cell can exclude or destroy most potential symbionts, so that a single dominant symbiont, which has managed to evade these defences, is typically present. Some kinds of intracellular parasite possess special techniques to gain access to cell interiors, while others simply allow themselves to be engulfed by macrophages, while contriving to avoid subsequent destruction. Enclosure of the symbiont by a vacuole can be thought of as tempering the severity of the extreme intracellular environment.

To give a final twist to the discussion of this complex and intriguing kind of biological association, M. H. Richmond discusses the bacterial cell as a habitat for extra-chromosomal DNA frag-

ments, such as plasmids, which can specify resistance to antibiotics or the ability to metabolise novel substrates. In this context plasmids can be regarded as molecular symbionts whose effect is to allow the bacteria which carry them to penetrate otherwise inhospitable habitats. □



A hundred years ago

THE first public act passed by the U.S. Congress during the present session, was one making an appropriation of 200,000 dollars for the construction, under the direction of the Secretary of the Treasury, for the National Board of Health of a vessel provided with suitable refrigerating apparatus, for the purpose of determining the possibility of destroying the yellow fever infection by intense cold. The act first introduced had special reference to the apparatus of Prof. Gamgee, but as passed it is within the power of the Secretary to select any device that will, in the opinion of the National Board of Health, best answer its purpose.

THE *Colonies and India* furnishes some interesting particulars respecting the so-called "vegetable ivory," which is now so much used as a substitute for ivory. The vegetable ivory nut is the produce of a species of palm found wild in South America and Africa. Inside the hard shell is the white kernel, which being softer than ivory and easily carved, as well as readily dyed, and being less brittle than bone, is largely used in making buttons, &c. The unripe fruit consists of a green shell, containing a watery fluid, which, as the nut ripens, gradually thickens until it becomes a pulpy mass, and eventually hardens into solid matter. The water, though bitter to the taste, is wholesome, and often renders invaluable service to travellers, who cannot otherwise obtain water to drink. The tree (*Phytelephas macrocarpa*) on which the fruit grows is unlike an ordinary palm, having little or no stem and drooping downwards, especially when the weak branches are overweighted by the six or seven bunches of nuts, each containing six or seven seeds, inclosed in thick heavy shells and outer sheath, and weighing altogether from 20 to 24 lbs.

EXTRAORDINARY finds of gold have lately occurred in the gold-fields of Dutch and French Guiana and are causing great excitement.

From *Nature* 20, 22 May, 88, 89; 1879.

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review article

Virological evidence for the success of the smallpox eradication programme

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Smallpox has prevailed throughout the populated world during the last four centuries but the World Health Organization's eradication campaign has now apparently interrupted transmission of the disease in the human population. The risk of natural re-introduction of the disease is negligible.

TEN years ago the worldwide incidence of smallpox was estimated to be about 10 million cases annually but since October 1977, when a case of smallpox was recorded in Somalia, there has been no naturally occurring smallpox reported throughout the world. Hopes that the case in Somalia would be the last instance of smallpox in the world were destroyed by the outbreak of smallpox in Birmingham, UK in August 1978. This caused considerable concern among the scientific community as well as the public because laboratory virus stocks appear to have been the source of infection. Although the focus of attention has been on measures to minimise the potential danger of variola virus stocks in laboratories, it is equally important for the world scientific community to examine whether variola virus has really become extinct in the human population and if so, what future surveillance and research on orthopoxviruses are required to ensure the permanent status of this phenomenon. I attempt in this article to present a summary of scientific data and review these problems.

The origin of smallpox

Ancient records in China and India imply that smallpox, easily recognised by the descriptions of its typical rash, high death rate and extensive transmissibility, was present in the eastern part of the Asian continent long before the Christian era. There are also records from that time of attempts to immunise susceptible persons with extracts of smallpox lesions (variolation).

As man is the sole host of variola virus and no animal reservoir exists, continuous transmission of variola virus in the human population required aggregates of susceptible individuals such as first occurred about 5,000–6,000 yr ago when agricultural development made it possible to support populations of more than 500 living together in one place (F. Fenner, personal communication). The disease apparently spread in all directions from the eastern part of Asia along with population movements due to trade, religious and political conflicts and exploration. The disease first appeared in Asia Minor in the early Christian era and around the same period southern Europe was infected. Smallpox reached Japan in the eighth century, the Americas in the sixteenth century, Siberia in the seventeenth century and Australia in the eighteenth century. Its devastating effect when first introduced into a new population has been well described. The disease probably originated from one place—eastern Asia—roughly 5,000 yr ago and has prevailed over the past four centuries throughout the populated world.

A finding inconsistent with this theory is that Rameses V of Egypt died of an acute disease in 1,100 BC which left pock-like

lesions on his mummified face. Electron microscopic examination of the pock lesions on this mummy might shed light on the diagnosis of the disease and the World Health Organization (WHO) and the Egyptian government are discussing this possibility. If Rameses V's illness was smallpox, this means that smallpox was already present in northern Africa at that time. The Roman, Egyptian, Babylonian, Assyrian and Persian civilisations have left considerable historical records, but there is no evidence indicating a smallpox epidemic in these areas around that time. Two hypotheses can be proposed; the disease may have been introduced from the East (commercial trade between Egypt and Asia was well established as far back as 1,100 BC) and, leaving no recognisable records of a smallpox epidemic, may have died out spontaneously; or, the source of Rameses V's disease could have been the African continent, perhaps Central Africa with which Egypt might have had communications along the River Nile. However, there is no way of determining which hypothesis is correct.

Prevalence of smallpox

At the end of the eighteenth century, Edward Jenner showed that material extracted from the lesions of cowpox would immunise against smallpox, and could replace variolation. During the following 150 years Jenner's 'vaccine' was gradually accepted by health services all over the world. However, over the years the virus strains used for vaccine production did not remain identical to those used by Jenner. By the 1950s, the existence of an effective vaccine and its coordinated use in vaccination programmes had resulted in the interruption of indigenous smallpox transmission in many countries in the temperate zones. Smallpox was, however, still prevalent in many of the tropical countries of Africa, South America and Asia. One reason for this was that the smallpox vaccine rapidly lost potency when exposed to tropical climates.

In the early 1950s a method of freeze-drying smallpox vaccine, so that it retained its potency for at least 4 weeks at 37°C, was adapted to large-scale production¹. This freeze-dried vaccine, stable in tropical climates without refrigeration, greatly enhanced the prospects for smallpox eradication.

In 1967 the WHO initiated an intensified global smallpox eradication programme. At that time, smallpox was endemic in 33 countries and cases were reported by 44 countries (Fig. 1). The campaign involved a massive investment of manpower, vaccine, technology and managerial skills. The total cash input from international assistance during the past 12 years is estimated to be about \$100 million. The essential strategy of the

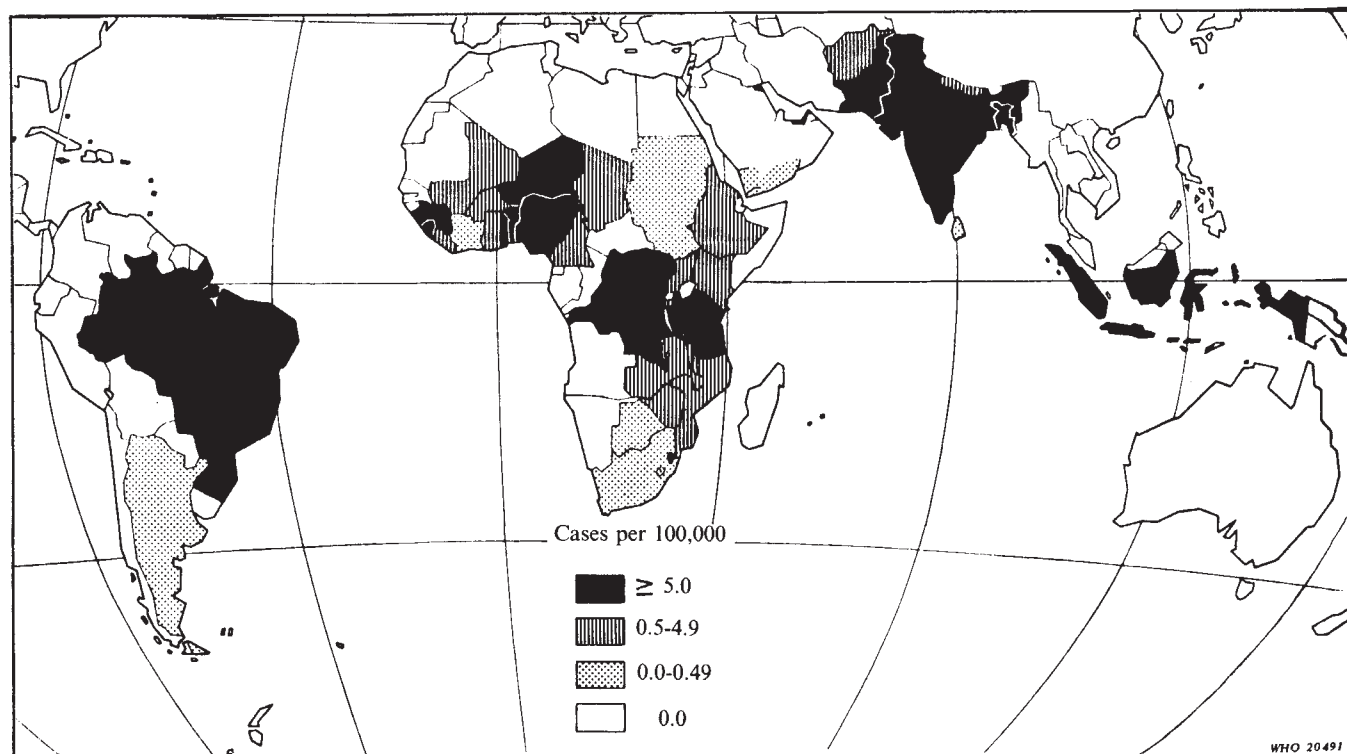


Fig. 1 Smallpox cases per 100,000 inhabitants in 1967.

programme was epidemiological surveillance combined with prompt containment of outbreaks detected².

Among milestones in the progress of the smallpox eradication programme have been the recording of the last cases in South America and West and Central Africa in 1971; in Indonesia in 1972; in southern Africa in 1973 and in the Asian subcontinent in 1975. Since 1976, the endemic foci were confined to the Horn of Africa. The last known case of smallpox of natural origin was detected in Merka town, southern Somalia in October 1977 (Fig. 2).

Certification of interruption of smallpox transmission

Do any foci of smallpox remain hidden in densely populated slum areas, remote tropical rain forests or isolated deserts? Past suspicions of this had some justification. A pockmark survey in Nigeria in 1971 revealed that the completeness of smallpox case reporting before the campaign began was only 1% in rural areas and 8% even in urban areas. In India, in 1973, when an active search for hidden foci was first carried out, it was revealed that less than 5% of all cases were reported in the entire country.

As the smallpox eradication campaign progressed, however, the efficacy of smallpox surveillance was considerably increased. National eradication programmes developed search operations for hidden foci even in the most remote areas, particularly when smallpox incidence in these countries became very low. However, there were a few exceptions. In Botswana an outbreak was detected six months after the last case was thought to have been found, and an outbreak in Indonesia followed a gap of 8 months in which no case was reported. Hence it was decided by the WHO Expert Committee for Smallpox Eradication in 1972 that active surveillance should be continued for at least 2 years after the last known case before eradication could be declared. The kind of major surveillance measures undertaken during this 2-year period in each of the countries concerned are illustrated by the following examples.

In India and Bangladesh, massive house-to-house searches were undertaken in the 2 years following the detection of the last

cases. On combining the data for the two countries it is found that an average of 98.2% of the 726,811 existing towns and villages were visited during each of the searches. Three such searches were conducted in India and eight in Bangladesh. No cases were found. Pockmark surveys were carried out in 28 countries of West, Central and southern Africa from 1975 to 1978. Of the total estimated population over 4% (9,059,119) were seen. No pockmarked person was found among children born after the year of the last known case in each country. In Ethiopia and Somalia, where the last endemic foci were detected during 1976 and 1977, the latest assessment results illustrate the intensity of the search for hidden foci; over 75% of the inhabitants had met search workers who enquired about smallpox rumours, over 75% knew about the reward which was offered to them if they found a smallpox case (Fig. 3), and over 70% knew where to report such a case.

The adequacy of all these activities has been verified independently during visits by an international group of experts (the International Commission for the Certification of Smallpox Eradication) convened by the WHO for certification purposes. Seventy-nine countries have been identified as either recently endemic for smallpox or exposed to the risk that an imported case of smallpox might establish endemicity. Of these, 64 countries have already been certified as free of smallpox and the remaining 15 countries are preparing for certification by the end of 1979 (Fig. 4).

Laboratory investigation of collected specimens

From 1973 to 1978, 11,249 specimens were collected from 51 countries—all situated in the tropical zone of Africa and Asia. All the specimens were tested by the WHO Collaborating Centres in Atlanta and Moscow with at least three standard methods—electron microscopic examination, virus isolation on chorioallantois of embryonated eggs and precipitation in gel testing. As the incidence of smallpox fell, so the hunt for residual foci intensified so that in 1977 and 1978, 8,507 specimens were tested.

Over 650 strains of variola viruses have been isolated, all of

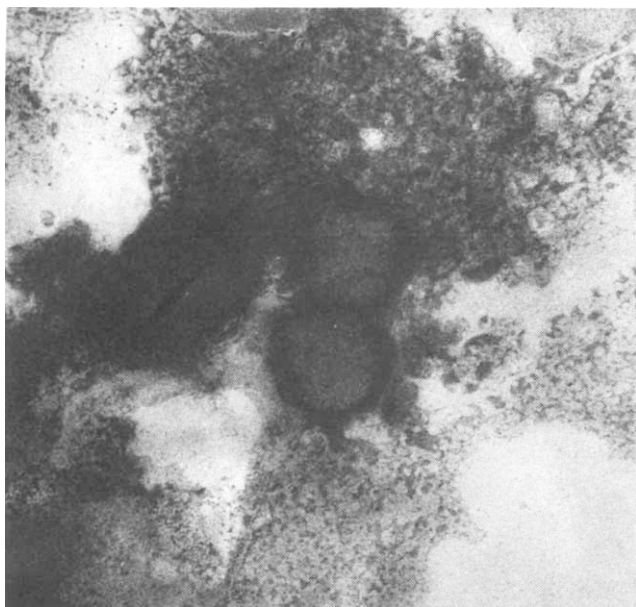


Fig. 2 Variola virus particles (variola minor) from a specimen taken from the last reported case, 26 October 1977.

which conformed with our previous understanding of the characteristics of this virus. Also, vaccinia virus (smallpox vaccine virus) has been isolated on several occasions, indicating that either the suspect patient had generalised vaccinia, or the specimens were contaminated with vaccinia virus. Monkeypox has been found in 36 sporadic cases of zoonosis in West and Central Africa (see below). And on one occasion, a poxvirus termed 'Lenny virus' was isolated from a person with rash and fever who died in eastern Nigeria in 1969³. This virus showed a mixture of the characteristics of variola and vaccinia virus, indicating a possible hybridisation of variola and vaccinia viruses during double infection in man. There was no evidence that this virus was transmitted among the inhabitants in the area.

Mild and severe smallpox

Laboratory examination of variola viruses isolated during the eradication campaign has shed light on the presence of different subspecies of variola virus. Since the report of the presence of mild smallpox 'Amaas' in southern Africa early this century, it has been recognised that there are two strains of variola virus, one, variola minor, causing fatality rates of less than 1% and the other, variola major, causing fatality rates of 20 to 40% among unvaccinated patients. The epidemiological investigation of 36,069 cases, including 2,444 deaths, during the past two decades indicated that fatality rates vary considerably according to the geographical areas; 15–36% on the Asian subcontinent, 8–13% in Africa and Indonesia, and less than 1% in South America⁴. Laboratory studies of more than 200 isolates obtained from these regions indicated that there were at least three sub-groups (A, B and C) in terms of haemadsorption tests on human embryo cell cultures infected by such isolates at 40 °C (ref. 5). Group A was dominant on the Asian sub-continent where the case fatality rate was highest, group B on the African continent where the case fatality rate was moderate, and group C in South America where it was the lowest. Thus, the conventional classification into variola major and minor appears to be inadequate; instead there might be a spectrum of variola virus strains varying considerably in their pathogenicity to man. Although this might indicate that the selection of a less pathogenic variola virus had occurred during long-established endemicity, it was essential for the global smallpox eradication

programme to exterminate all variola virus, regardless of its pathogenicity to man.

The possibility of variola virus in animals

In the literature from 1767 to 1949 there are seven references to a smallpox-like disease in non-human primates⁶. Except for the one in Indonesia, which occurred in the Jakarta Zoo in 1949, there was no valid evidence that any of these was true smallpox. Between 1966 and 1970, 7,497 blood and tissue specimens collected from mammals in West Africa were examined for various viruses⁷. Of the specimens, 104 were from non-human primates and 5,517 were from rodents. Among 83 virus isolates obtained there was only a single pox virus (gerbilpox) and it was distinguishable from variola virus. From 1970 to 1975, 1,024 sera were collected from non-human primates from tropical Asia without obtaining any evidence of poxvirus infection⁸.

More important are the epidemiological findings. Notably, in the Philippines and Central America, where smallpox has been eradicated for several decades, it has never returned spontaneously, although in these areas non-human primates are plentiful. Also, during the last 10 years of the smallpox eradication campaign, comprehensive epidemiological investigations were conducted on more than 50,000 outbreaks in South America, Africa and tropical Asia. None of the sources of infection could be traced back to an animal origin.

Orthopoxviruses in animals

Variola virus is a member of the genus *Orthopoxvirus*. Six important viruses in this group are presented in Table 1. All these viruses have close antigenic relationships. Vaccinia, cowpox and camelpox viruses lack continuous transmissibility in man, but the situation with monkeypox virus and whitepox virus deserves further comment.

Monkeypox virus: In 1958 a poxvirus was isolated from an outbreak of pox disease in a captive monkey colony in a laboratory in Copenhagen⁹. The virus was called monkeypox virus. The WHO, in surveys on 27 laboratories from 11 countries conducted in 1968 and 51 laboratories from 25 countries in 1970, came up with 10 previous monkeypox outbreaks in laboratories. In one instance monkeypox virus was isolated from a giant ant-eater which temporarily shared a cage with an infected monkey. No monkeypox infection has been reported in laboratories since 1969. No human infections have been associated with any of these outbreaks but the vaccination status of those in contact with the monkeys is not known.

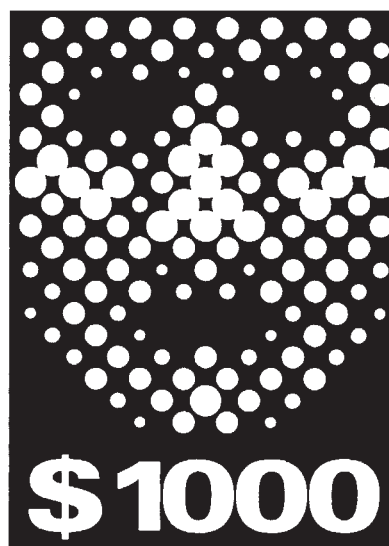


Fig. 3 "The World Health Organization offers \$1,000 to the first person reporting an active smallpox case resulting from human-to-human transmission and confirmed by laboratory tests. Valid until global eradication is certified."

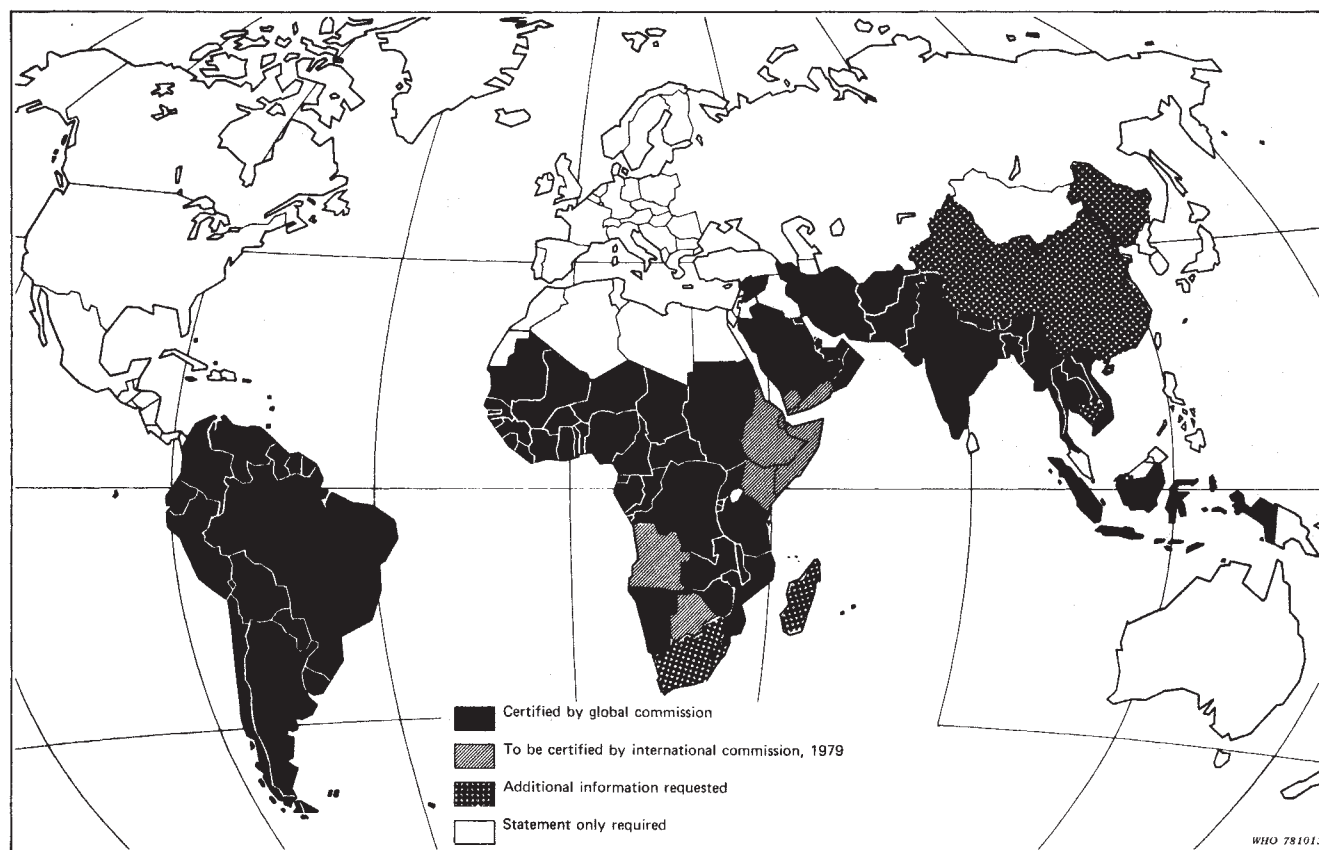


Fig. 4 Global certification of smallpox eradication by the end of 1979.

Monkeypox has not been reported in wild monkeys. The virus was considered a laboratory curiosity until 1970 when the first case of human disease was discovered in remote tropical rain forest of Equateur Region, Zaire^{10,11}. From 1970 to 1978, 36 cases of human monkeypox were reported from West and Central Africa. In Zaire, 27 cases have occurred, in Liberia, four, in Nigeria, three, and in the Ivory Coast and Sierra Leone, one each. All the cases have clinically resembled smallpox. Six persons have died, a mortality rate (16%) similar to smallpox in West and Central Africa. Only four patients had a vaccination scar. Most cases occurred in the zone of tropical rain forests where people are hunters and have frequent close contact with a variety of domestic and wild animals (Fig. 5).

There has been no evidence of person-to-person transmission except in two families where second cases occurred 8 and 12 days after the first. These cases suggest secondary transmission unless separate exposures to a common source of infection occurred. Assuming secondary transmission did occur, the potential for interhuman spread of monkeypox can be estimated from the number of susceptible family and community members (those without a smallpox vaccination scar) who had close contact with patients having active disease. Of 56 susceptible contacts, only two (3.6%) came down with the disease. This secondary attack rate is 10 times lower than the transmission rate of 35% for smallpox estimated in West Africa. Tertiary spread of human monkeypox has never been reported.

Special vaccination scar and facial pockmark surveys were carried out in 1975 in populations living near where human monkeypox cases had occurred 4 to 5 years previously in the Ivory Coast, Liberia, Nigeria and Sierra Leone. A relatively low immunity level was found in younger age groups. There were no vaccination scars in 57% of 2,125 children aged 0–4 yr and 29% of 8,047 school-age children, indicating their probable susceptibility to monkeypox infection. No evidence of monkeypox (or smallpox) was found.

The source of human monkeypox infections is as yet unknown. Recent serological studies conducted in the immediate areas where human monkeypox cases occurred have shown a 23% (50/125) prevalence of poxvirus-neutralising antibodies in non-human primates¹². Of these, three sera showed monkeypox-specific antibody¹³. No human monkeypox has been reported from South America or Asia. Little is known about its natural history, possible animal reservoir or pattern of transmission. Thus, although monkeypox does not appear to frustrate the achievement of smallpox eradication, further research on monkeypox in West and Central Africa with special emphasis on Zaire is warranted. It will be supported by the WHO, for whom the clinical resemblance of the disease to smallpox is one of the problems encountered in its continuing surveillance for smallpox.

Whitepox virus: Although attempts to isolate monkeypox virus from animals captured near human monkeypox cases have failed, four whitepox virus isolates have been obtained during laboratory examination from 1970 to 1978. These wild whitepox strains came from the kidney tissue of one chimpanzee, one wild monkey and two rodents all captured in Equateur Region of Zaire⁸. Before this, two strains of whitepox virus had been isolated from routine monkey kidney cell cultures in 1964–65 in another laboratory. The monkeys had come from Malaysia. So far laboratory tests have been unable to determine the nature of these isolates or to distinguish them from variola virus. Note, however, that there has been no evidence of human infection by the virus in the areas where animals for specimen collection were captured.

In 1978, a laboratory reported that cloned variants of some monkeypox virus strains showed the characteristics of whitepox virus and that in hamsters inoculated with a monkeypox virus strain, monkeypox virus was isolated at an early stage of infection but a few whitepox virus isolates were made at a late stage of infection^{14,15}. However, the experiences of several other

Table 1 Comparison of some orthopoxviruses

Characteristics	Variola	Whitepox	Monkeypox	Vaccinia	Cowpox	Camelpox
Isolated from	Man	Ape, monkey rodent	Man, monkey anteater	For vaccine production, origin unknown	Man, cow, large felines	Camel
Pocks on chorioallantois of chick embryo	Small, white	Small, white	Small, pink	Large, white to grey	Haemorrhagic	Small, white
Ceiling temperature on chorioallantois of chick embryo (°C)	37.5–38.5	38.5	39	41	39.5	38.5
Growth in rabbit skin	—	—	++	+ or ++	++	+
Pathogenesis for baby mice	Low	Low	High	High	High	Low
Antigens specific to:						
Vaccinia	—	—	—	+	+	+
Variola	+	+	—	+	?	+
Monkeypox	—	—	+	—	?	—
Polypeptide pattern	Character of variola	Character of variola	Character of monkeypox	Character of vaccinia	Character of cowpox	?
Thymidine kinase sensitivity	+	+	—	—	—	—

investigators have produced conflicting results, namely that white pock variants of monkeypox have not been similar to whitepox virus. Further experiments to clarify these two sets of different observations are now under way in two WHO Collaborating Centres.

Although laboratory studies on whitepox virus have proved somewhat confusing, epidemiological evidence certainly suggests that whitepox virus must differ from variola virus in some way because: (1) no human infections with a variola-like virus have occurred in Malaysia during the last 12 years or in Zaire during the 7-year period since the last smallpox cases were recorded; and (2) it can be presumed that the surveillance system, particularly in Zaire, is sensitive enough to have detected any infection with a variola-like virus as shown by the capability to detect human monkeypox. Nevertheless, this virus merits continued laboratory and field investigations.

Mutation of poxviruses to variola virus

History suggests that smallpox arose in one area and spread across the world from the initial focus. Although other orthopoxviruses appear to be widespread in nature, there is no evidence of any other 'spontaneous' appearance of smallpox. This is strong evidence against the hypothesis that other poxviruses might give rise to variola virus by mutation.

Persistence of virus in convalescence and on fomites

Variola virus can be recovered from the urine or nasopharynx of infected patients for not more than 25 days after the onset of the rash¹⁶. This finding is consistent with epidemiological experience that recovered patients are no longer infectious.

The possibility of smallpox scabs remaining in the houses of patients in previously endemic areas has caused concern, as in 1968 it was reported that scabs kept at room temperature on a shelf in a laboratory in a temperate zone for over 13 years contained viable virus¹⁷. More recent experiments, however, measuring virus decay in tropical conditions, indicate that virus concentration in scabs may decrease to a non-infective level within three weeks¹⁸.

The practice of variolation persisted until very recently in some remote tropical areas of Africa and Asia. The last known use of variolation was in Ethiopia in 1976. Samples of variolator's stocks have been collected from Afghanistan, Ethiopia and Pakistan during the past 10 years. Of 21 specimens, 17 did not grow variola virus despite the fact that several specimens showed numerous poxvirus particles on electron microscopy.

Four specimens, all from Afghanistan were positive for viable virus when tested 4–9 months after the variolators had collected their material, but Afghanistan has maintained freedom from smallpox for 5 years; the last case was recorded in 1973. In Ethiopia, once smallpox outbreaks stopped, the practice of variolation also ceased.

Conclusions

Current evidence from field and laboratory investigations indicates convincingly that when the interruption of smallpox transmission in the human population has been confirmed throughout the world, the risk of re-introduction of the disease will be negligible. This assessment may logically lead to the universal termination of routine smallpox vaccination programmes. This, however, should be accompanied by continuing studies and vigilance. The surveillance of orthopoxvirus

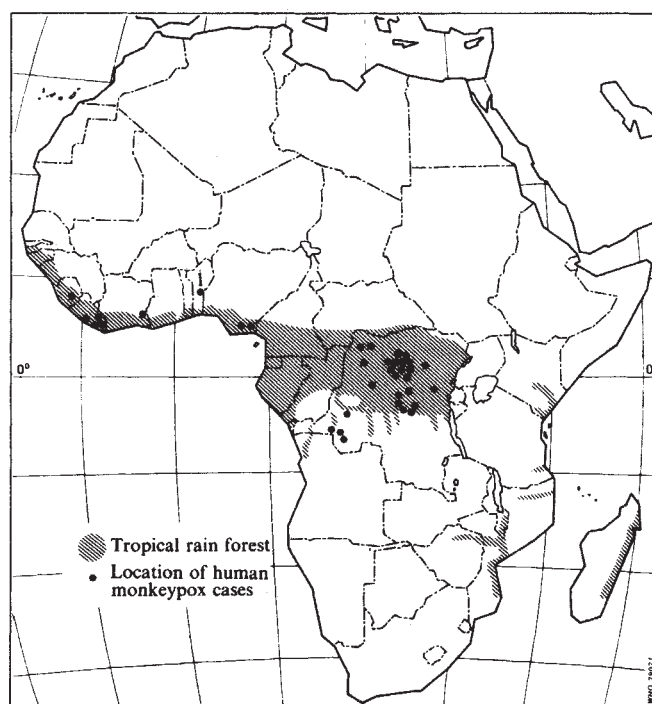


Fig. 5 Location of human monkeypox cases 1970–78. (1, Patient reported to a hospital in Benin, but developed pockmarks in western Nigeria.)

infections in animals and man as well as laboratory studies of orthopoxviruses should continue and all necessary steps should be taken to minimise the potential danger of live variola virus stocks. To this end, the first meeting of the Global Commission for the Certification of Smallpox Eradication was convened by the WHO in December 1978 and it made comprehensive recommendations in relation to surveillance and research after certification of smallpox eradication, which is expected to take place by the end of 1979.

I thank Professor Keith Dumbell, Dr D. A. Henderson and John Wickett who gave advice, and all colleagues in many countries who provided substantial data for the assessment of the smallpox eradication programme.

Note added in proof: Since submission of this article smallpox eradication has been certified in six more countries: Angola, Botswana, Iraq, Lesotho, South Africa and Swaziland. Thus there remain only nine countries which are preparing for certification before the end of 1979.

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articles

Sm–Nd dating of Onverwacht Group Volcanics, southern Africa

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Sm and Nd were unaffected by alteration of the Onverwacht lavas permitting for the first time a precise Sm–Nd date ($3,540 \pm 30$ Myr) to be obtained for this major igneous event.

THE Onverwacht Group Volcanics, occurring in Swaziland and the Transvaal of South Africa, are probably the most extensively studied Archaean greenstone belt volcanics^{1–7}. Ultramafic volcanic rocks were first well documented from the Onverwacht^{1–3} and have been generally termed komatiites⁴. Much of the vigorous debate concerning the nature of the Earth’s first stable crust has centred on comparisons of structural, metamorphic and geochemical features of the Onverwacht Group and the Ancient Gneiss Complex^{1,5–7}, the oldest element of the granitic terrain surrounding the Swaziland Supergroup.

The Onverwacht Group forms the basal part of the Barberton greenstone belt and together with the overlying sedimentary Fig Tree and Moodies Groups constitutes the Swaziland Supergroup. The Onverwacht Group itself consists of two distinct volcanic units¹. One of these, the lower ultramafic unit (LUU), is separated from the other, the mafic-to-felsic unit (MFU) by a persistent sedimentary band termed the Middle Marker Horizon. Each unit is subdivided into three formations which from base to top are the Sandspruit, Theespruit and Komati and the Hoogenoeg, Kromberg and Swartkoppie Formations¹. The

LUU consists mainly of peridotitic and basaltic komatiites with subordinate basic and acid tufts, and small, generally concordant, penecontemporaneous, felsic porphyry intrusions². The MFU is of less mafic character and has a greater abundance of chemical sediments³.

Despite the considerable geological importance of the Onverwacht Group, geochronological studies have been unsuccessful in either precisely defining the time of the volcanism or establishing the temporal relationship between the Swaziland Supergroup and the Ancient Gneiss Complex. Whole rock Rb–Sr studies⁸ of the latter indicate repetitive metamorphism and Sr isotope rehomogenisation for ~200 Myr after initial formation of the gneisses ~3,300 Myr ago ($^{87}\text{Rb}/^{86}\text{Sr} = 1.42 \times 10^{-11} \text{ yr}^{-1}$). The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7006 ± 24 (all quoted errors are at the 95% confidence level ($26 \sigma_m$)) for those gneisses at 3,300 Myr when compared with probable mantle values during the Archaean constrain a single-stage crustal prehistory of their parent materials to a period of no more than 100–150 Myr.

Greenschist facies metamorphism which pervades the Swaziland Supergroup^{1,9} has perturbed the Rb–Sr systematics such that they now give conflicting impressions as to the age of volcanism. For example, whole-rock Rb–Sr data¹⁰ from the felsic volcanics suggest an age of $2,570 \pm 40$ Myr, whereas Rb–Sr results for whole rock mafic volcanics provide no useful geochronological information^{10,11}. The Rb–Sr ages obtained on sediments of the Swaziland Supergroup are $3,280 \pm 70$ Myr for

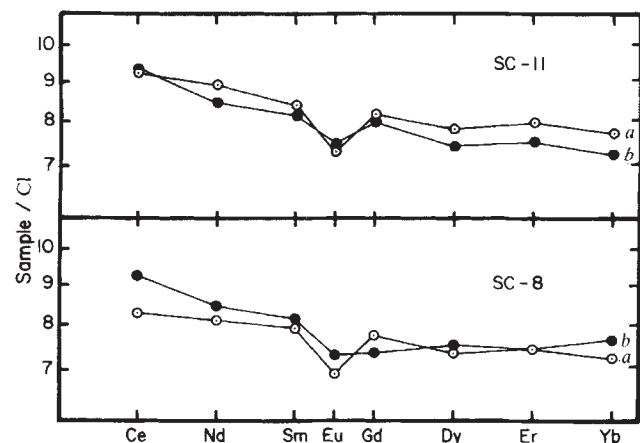


Fig. 1 C1-chondrite normalised²³ abundance patterns for two pillow margin(a)–interior(b) pairs.

the Middle Marker Horizon¹² and $2,920 \pm 20$ Myr for the Fig Tree Group¹³. These results provide minimum times for the deposition of the sediments and indicate that the Rb–Sr data for the felsic volcanics reflect metamorphic resetting at 2,570 Myr (ref. 10). The U–Pb¹⁴ results obtained on zircon and sulphide separates from felsic volcanics of the MFU yield a slightly older age than the Rb–Sr data at $\sim 3,310$ Myr. Furthermore, an internal isochron obtained from mineral density separates of a basaltic komatiite from the Komati Formation yields a Rb–Sr age of $3,430 \pm 200$ Myr and a well defined initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.70048 ± 5 (ref. 11).

Despite considerable effort, adequate geochronological data have not yet been obtained on the Onverwacht volcanics. We present here the first definitive age for the Onverwacht volcanism obtained from Sm–Nd analyses of whole-rock samples.

REE abundances

The difficulty of obtaining reliable ages from the Onverwacht volcanics using a parent–daughter system which involves an alkali and an alkaline earth element is not surprising in view of the evidence for element mobility obtained from volcanic rocks which have suffered low-grade alteration^{15–22}. Both Condie *et al.*²² and H.S.S. and A.J.E.²⁰ have investigated the effects of metamorphism on the chemistry of Onverwacht volcanics. Whereas H.S.S. and A.J.E.²⁰ have made detailed investigations of pillow lava margins and interiors, Condie *et al.*²² have studied the variations within individual massive flows. Both studies, however, indicate variable fractionation of Rb/Sr during alteration and also demonstrate labile behaviour for K, Na, Ba, S, Cu and Zn, for example. Condie *et al.*²² have further suggested that the light rare-earth elements (REE) have migrated relative to the heavy REE, although the evidence for such a conclusion is sparse.

Table 1 REE abundances for two LUU pillow lavas

	SC-8		SC-11	
	Margin (a)	Interior (b)	Margin (a)	Interior (b)
Ce	10.3	11.5	11.5	11.6
Nd	7.3	7.6	8.0	7.6
Sm	2.21	2.27	2.34	2.27
Eu	0.72	0.76	0.76	0.78
Gd	2.74	2.60	2.90	2.83
Dy	3.13	3.21	3.34	3.16
Er	2.02	2.02	2.17	2.05
Yb	1.88	1.99	2.01	1.89

To further assess the possibility of differential behaviour of REE during the alteration of Onverwacht samples, REE abundances have been determined using precise isotope dilution techniques²³ for two pillow margin–interior pairs previously studied by H.S.S. and A.J.E.²⁰. These REE data are presented in Table 1 and plotted relative to average C1 chondrite abundances²³ in Fig. 1. The small differences that exist within each pillow can be readily attributable to differences in phenocryst content and provide little evidence, in this particular instance at least, for differential movement of REE during low-grade alteration. Note that these particular pillow lavas show variations in Rb/Sr between margin and interior of between 20% and 400%²¹. The successful application^{24–27} of the Sm–Nd system to the dating of Archaean igneous rocks of diverse metamorphic grade in general militate against metamorphic disturbance of Sm–Nd systematics.

Sm/Nd ratios

The samples used in this study are from the type areas of the Theespruit (LUU), Komati (LUU) and Hoogenoeg (MFU) Formations, and were selected for optimum spread in Sm/Nd ratio coupled with preservation of original textures and relict

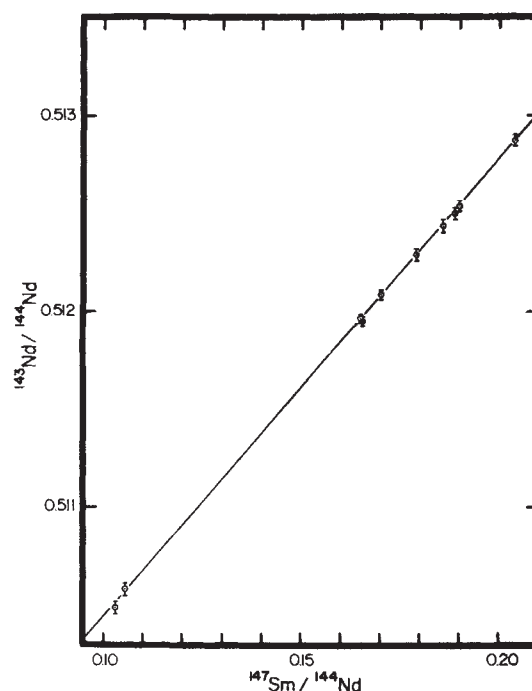


Fig. 2 Sm–Nd evolution diagram for the samples from the lower ultramafic unit. The data yield an age of $3,540 \pm 30$ Myr and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50809 ± 4 with errors quoted at the $2\sigma_m$ level.

mineral content. None of the samples is totally fresh and typically, olivine is serpentinised, plagioclase sericitised and glass devitrified. Other common secondary minerals include calcite, tremolite, epidote, chlorite and talc. Sm and Nd concentrations and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios were determined (see refs 24 and 32 for analytical details) for 10 samples from the LUU and two from the MFU (Table 2). Sm–Nd data for samples from the LUU, which range from acid pyroclastics to spinifex-textured ultramafics, are plotted on a Sm–Nd evolution diagram in Fig. 2. The best-fit regression line²⁸ through the data points corresponds to an age of $3,540 \pm 30$ Myr and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50809 ± 4 . ($^{147}\text{Sm}\lambda = 6.54 \times 10^{-12} \text{ yr}^{-1}$). The data for the seven mafic and ultramafic samples alone yield an age of $3,510 \pm 60$ Myr and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50813 ± 7 which are not significantly different from those obtained from all the LUU data.

Table 2 Sm-Nd data for Onverwacht Group Volcanics

Sample	Rock type	Formation	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}^*$	$^{143}\text{Nd}/^{144}\text{Nd} \pm 2\sigma_m$
Lower ultramafic unit						
HSS-74	Sodic porphyry	Komati	2.129	12.42	0.1030	0.510487 ± 36
HSS-161	Acid tuff	Theespruit	2.033	11.59	0.1054	0.510570 ± 32
HSS-52B	Felsic pillow lava	Komati	4.891	17.79	0.1653	0.511950 ± 22
HSS-56	Basaltic lava	Komati	1.514	4.464	0.2040	0.512875 ± 32
R-14	Basaltic komatiite	Komati	1.115	3.551	0.1888	0.512504 ± 34
HSS-32	Basaltic komatiite	Komati	3.390	13.06	0.1649	0.511957 ± 22
HSS-88A	Peridotitic komatiite	Komati	1.267	4.251	0.1792	0.512292 ± 34
HSS-92	Peridotitic komatiite	Komati	0.5750	1.861	0.1858	0.512439 ± 34
HSS-95	Peridotitic komatiite	Komati	0.5490	1.736	0.1902	0.512541 ± 28
HSS-523	Peridotitic komatiite	Komati	1.318	4.659	0.1701	0.512084 ± 20
Mafic to felsic unit						
HSS-224	Tholeiitic basalt	Hoogenoeg	4.316	14.04	0.1849	0.512478 ± 22
19-J	Acid pyroclastic	Hoogenoeg	2.154	11.51	0.1125	0.510717 ± 24

* $^{147}\text{Sm}/^{144}\text{Nd}$ ratios are determined to a precision of 0.2% at the $2\sigma_m$ level.

Inclusion of the data for the two samples from the MFU, though not plotted in Fig. 2, results in a less precise but indistinguishable age of $3,570 \pm 50$ Myr and an initial $^{143}\text{Nd}/^{144}\text{Nd}$ of 0.50807 ± 6 . The Sm-Nd data satisfy the normal criteria for designating the regression line an isochron. These results do not resolve the time interval, represented by the Middle Marker Horizon, between the volcanic activity of the LUU and the MFU¹.

If a single-stage mantle source history is assumed for the LUU volcanics from 4,550 Myr until 3,540 Myr ago and the $^{143}\text{Nd}/^{144}\text{Nd}$ ratio 4,550 Myr ago was equal to that of the Angras dos Reis achondrite (0.50682)²⁹ then the time-integrated Sm/Nd ratio for this interval was 0.297 ± 0.005 . This value is within error of those estimated from whole-rock isochron studies of the Isua²⁵, Rhodesian²⁴ and Monroe Township²⁷ greenstone belt volcanics (Table 3) and together support the conclusion of DePaolo and Wasserburg^{30,31} that Archaean mantle evolved with approximately chondritic Sm/Nd.

The peridotitic komatiites represent large degrees of partial melting whose primary abundance ratios of large ion lithophile elements may closely approximate those of their source region. Four samples of this rock type analysed in this study have Sm/Nd ratios which range from 0.270 to 0.302 and are within about 10% of the time-integrated Sm/Nd ratio of their source.

Conclusions

Reliable initial $^{87}\text{Sr}/^{86}\text{Sr}$ data for the Onverwacht volcanics could not be obtained from any of the samples available²¹. However, if the precise initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio determined by Jahn and Shih¹¹ for a LUU basaltic komatiite is assumed to be that characterising the LUU source region 3,540 Myr ago then an estimate of the time-integrated Rb/Sr ratio of the source can be obtained in a manner analogous to that used for the Sm/Nd ratio. The calculated time-integrated Rb/Sr ratio is $0.034 \pm$

0.001, which is similar to that of 0.03 suggested previously^{31,32} on the basis of the correlation of Nd and Sr isotope compositions in oceanic basalts.

Resolution of the temporal relationships between the Onverwacht Group and the Ancient Gneiss Complex must await further geochronological data for the latter which are of comparable precision to those reported here for the Onverwacht. Although the Onverwacht volcanics are of similar age to the Amitsoq gneisses³³⁻³⁵ of West Greenland they do not represent the oldest known basic crust. The Isua metavolcanics which have been precisely dated by both U-Pb (ref. 35) and Sm-Nd methods²⁵ are some 200 Myr older.

We thank G. Wesselmann for skilled technical assistance. This work was supported by NSF grant EAR 75-20891.

Received 16 January; accepted 20 March 1979.

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Table 3 Sm-Nd Ages, initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios and inferred time-integrated (ti) Sm/Nd ratios

Rock suite	Age (Myr)*	Initial $^{143}\text{Nd}/^{144}\text{Nd}^*$	(Sm/Nd) _{ti} *
Isua ²⁵	$3,770 \pm 40$	0.507831 ± 46	0.306 ± 0.006
Onverwacht	$3,540 \pm 30$	0.50809 ± 4	0.297 ± 0.005
Lewisian ²⁶	$2,920 \pm 50$	0.508959 ± 49	0.311 ± 0.003
Rhodesia ²⁴	$2,640 \pm 140$	0.50919 ± 18	0.294 ± 0.010
Monroe Township ²⁷	$2,655 \pm 43$	0.50924 ± 6	0.304 ± 0.003

* Errors quoted at $2\sigma_m$.

Hydrogen production from coal, water and electrons

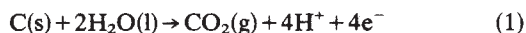
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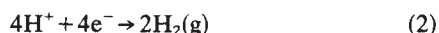
Coals and other forms of solid carbonaceous fossil fuel are oxidised to oxides of carbon at the anode of an electrochemical cell and hydrogen is produced at the cathode, these gases being produced in relatively pure states. The reaction proceeds at very mild temperatures and at operating electrical potentials significantly lower than the thermodynamic potential of water electrolysis. Although the reaction is readily observable at room temperature, the observed activation energies and the expected decomposition temperatures of the presumed intermediates suggest that much more rapid and steadier oxidation rates might be achieved at higher temperatures in the range 200–600 °C.

FOSSIL fuels and water are the major raw materials used to manufacture hydrogen although electrical energy produced by nuclear fission may become important for future production of hydrogen by electrolysis. Hydrogen is a key chemical for purifying petroleum of environmentally polluting ingredients; it is also important for converting the solid fossil fuel coal into clean fluid fuels. Mills¹ has specified hydrogen as the most costly ingredient in such conversions, the chemistry and technology of which are reviewed elsewhere^{1,2}

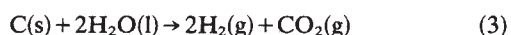
We report here a newly developed electrochemical process which converts coal and water into two separate gaseous products: one comprising essentially gaseous oxides of carbon and the other essentially pure hydrogen. The process chemistry takes place at mild temperatures (even room temperature) and the gaseous products are essentially free of impurities such as ash, tar and sulphur compounds. This new electrochemical gasification process involves the anodic oxidation of coal at an electrode for which we postulate the half-cell reaction of the carbon in coal:



in combination with a corresponding half-cell reaction at the cathode:



The net sum of these half-cell reactions (1) and (2) is the equation for the predominant reaction in the electrochemical gasification of coal:



This electrochemical gasification process, instead of producing a complex mixture containing H₂, CO, CO₂ and impurities (as in conventional steam-carbon gasification), produces relatively pure streams of carbon oxides in the anode compartment and hydrogen at the cathode.

In principle, conventional water electrolysis requires a theoretical thermodynamic electrical energy input [$\Delta F^\circ = -nFE^\circ$] of 56.7 kcal per mol of hydrogen and a corresponding theoretical driving potential of about 1.23 V whereas electrochemical gasification (equations (1)–(3)) requires according to thermodynamic principles only about 9.5 kcal of electrical energy and a reversible potential of only 0.21 V to produce 1 mol of hydrogen. The greatly lowered electrical energy requirement for the latter process results, of course, from the consumption of coal which can be viewed as supplying the additional electrons required by the process.

Of course, the stoichiometric reactions written above mask reaction mechanism(s) which as yet can only be speculative. Perhaps the primary electrode process is the discharge of OH[−] at the anode to produce a radical which attacks the coal. Overall, the carbon of the coal might also be viewed as an oxygen acceptor.

Conventional production of hydrogen from coal uses steam-carbon reactions to produce synthesis gas (CO and H₂) by a strongly endothermic reaction which must be conducted at temperatures sufficiently high (~800 °C) to assure favourable equilibrium. Synthesis gas must then be purified to remove sulphur compounds and other impurities followed by reaction with water to shift the CO/H₂ ratio as required. Coal gasification technology is discussed in detail elsewhere^{2,3–5}.

We have conducted electrochemical gasification of three coals, one lignite and one char (obtained from the Pittsburgh Energy Research Center of the US Department of Energy) by anodic oxidation in the apparatus of Fig. 1. Typical results recorded in Table 1 show substantial oxidation currents at applied potentials significantly lower than those necessary to electrolyse aqueous electrolytes, compared with control experiments made in the absence of coal. Hydrogen was produced at the cathode and carbon dioxide containing 3–7% carbon monoxide at the anode. Oxidation of the coal apparently requires contact with the anode—only background current is measured if the anode is surrounded by a porous membrane which prevents the close approach of the coal particles but permits transport of species dissolved in the anolyte.

In these early experiments, Montana Rosebud char gave higher current and seemed easier to oxidise anodically than the parent coal. Based on other experiments with active carbon, diamond and graphite we attribute this behaviour to the higher surface area, smaller particle size and more graphitic nature of the char as compared with the parent coal. Susceptibility of a carbonaceous particle to anodic oxidation may be related to the electrical conductivity of the particle.

Figure 2 shows the inter-relationship of oxidation current, temperature and coal-to-electrolyte concentration; increasing such concentrations or temperature causes current to increase as

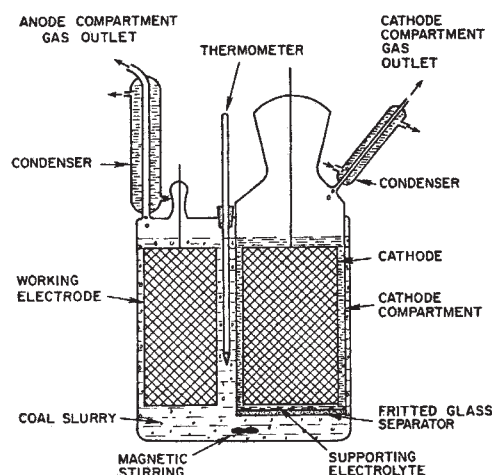


Fig. 1 Stirred slurry gasification cell. Supporting electrolyte H₂SO₄, 475 ml volume. Anode and cathode were Pt gauge 52 mesh with respective areas of 96.5 cm² and 158 cm².

Table 1 Comparison of oxidation rates of different coal samples

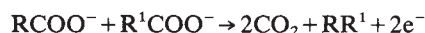
Coal	Potential of oxidation (V)	Coal concentration (g cm ⁻³)	Oxidation rate (mA)
Montana Rosebud char	0.78	0.475	8.20
North Dakota lignite	0.83	0.475	9.00
Pittsburgh coal	0.875	0.475	5.30
Illinois no. 6	0.875	0.475	3.00
Montana Rosebud coal	0.78	0.475	1.50

Electrolyte, 3.7 M H₂SO₄; temperature, 23 °C; electrode area, 6.5 cm²; coal slurry concentration, 0.475 g cm⁻³; particle size, 250 µm and below; experimental apparatus as in Fig. 1. Control experiments with no coal, lignite or char produced currents of only about 0.05 mA.

expected. The temperature dependence of oxidation rate leads to an estimate of apparent activation energy of about 10–12 kcal mol⁻¹ for North Dakota lignite at 1 V. Such low activation energy suggests that the rate determining step may not be a typical chemical reaction. Particle size effects have also been observed but they are complex and seem to be time dependent. The ash content, porosity and other properties of the particles may be size dependent and particle size is also related to momentum available for a particle to penetrate the nerstian layer at the anode. We have also obtained comparable results with electrodes other than Pt (such as graphite) and other electrolytes (such as HCl). The higher the potential the greater the oxidation current. As the coal is consumed by oxidation at a given potential the current diminishes as shown in Fig. 3 where current is plotted against the potential for various extents of coal consumption as a parameter. Anodic oxidation of carbon anodes has been investigated previously⁶. The controlled oxidation of purer carbons, whether by electrochemical or chemical means, results in the formation of surface oxides such as hydroxyl, carbonyl or carboxyl groups^{6–8}. We believe that such oxides also form during the anodic oxidation of coal and, as they increase in concentration on the surface of the coal particles, the potential required for additional oxidation increases. These surface oxides of carbon decompose to gaseous oxides of carbon on heating and this is consistent with the experimental observation that as temperature increases lower potentials are required to produce the same rate of oxidation. The gradual decrease of the reaction rate may be attributed to a corresponding accumulation of chemisorbed oxygen in the form of surface functional groups on the coal^{6–8}. Based on the known decomposition temperatures⁹ for such surface compounds it should be possible to maintain a higher and steady oxidation rate at 200–600 °C, perhaps even at applied potentials lower than those reported here. Of course, such higher temperatures would entail operation at pressures above atmospheric.

Some experiments were performed in which, after significant consumption of the coal had taken place by anodic oxidation at about 100 °C, and the anodic current at 1 V had decreased to low values, the coal was removed from the electrolyte and heated to about 200 °C to decompose surface oxides. When this coal was returned to the anolyte, high oxidation currents were measured at 1 V again, and these currents were almost equal to those provided by virgin coal at the same conditions. Reactivity could also be restored by extracting reacted coal with hydrocarbon solvents.

Surface carboxyl groups on coal or smaller carboxylated fragments can probably react through a Kolbe-type pathway to form aliphatic type hydrocarbons:



Surface free radicals, R·, might also form by



Such reactions may be responsible for the tar-like substance that can be extracted from anodically oxidised coals using hydrocarbon solvents. For example, acetone extracted about 5% of the weight of 25%-consumed NDl to form a dark, reddish-

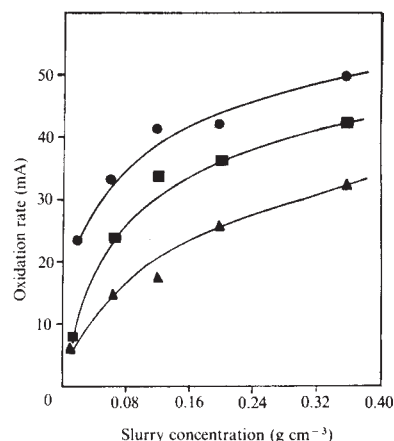


Fig. 2 Effect of coal concentration on the oxidation rate. (North Dakota lignite; particle size, 44 µm and below; supporting electrolyte concentration, 4.13 M H₂SO₄; oxidation potential, 1.00 V; experimental apparatus as in Fig. 1 except anode area 6.5 cm². ●, 78 °C; ■, 59 °C; ▲, 39 °C.

brown solution, whereas a similar treatment of the parent coal produced an extract that remained almost clear.

Gases produced and the current efficiency

During oxidation of ND lignite at 114 °C and at potentials near 0.85–1.0 V the gas produced at the cathode was essentially pure H₂. Based on 12 experiments in each of which about 100 cm³ of H₂ was collected, the mean current efficiency (defined as: mol H₂ collected/mol H₂ corresponding to coulombs passed) was 1.00 with a standard deviation of ±0.022. The gas produced within the anode compartment was almost pure CO₂ with small amounts of CO ranging from about 7% early in the electrolysis (about 1,870 C passed, equivalent to about 0.34% of coal consumed) to a steady-state value of about 3% attained at about 3,740 C passed (equivalent to about 0.68% of coal consumed). The volume ratio of the gases collected at the cathode to those at the anode ranged from ~3.5 to ~8; the higher ratios were obtained at the beginning of the experiment but then decreased. According to reaction (3) the gas ratio should be about 2. In the present conditions, however, a significant portion of the carbon oxides presumably remained bound to the coal (probably as -COOH, -CHO and -CH₂OH groups) and this would account for the gas volume ratios >2. The decrease in these ratios as the experiment progresses may be attributed to the build-up of oxygen on the carbon surface until a steady-state saturation is reached. Higher temperatures should cause more oxides of carbon to be liberated from the coal with an attendant lowering of the ratio of cathode-to-anode gas production rate. Some electrolyte-soluble, (probably oxygenated) organic compounds

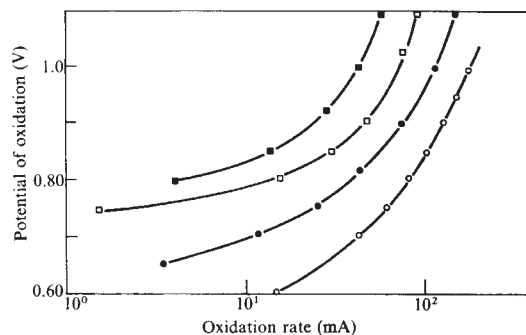


Fig. 3 Effect of potential on the oxidation rate as the reaction proceeds. (North Dakota lignite; coal slurry conc., 0.069 g cm⁻³; supporting electrolyte, 5.60 M H₂SO₄; particle size, 125–149 µm; temperature, 114 °C experimental apparatus, as in Fig. 1 with anode area 96.5 cm². Total carbon consumed: ○, 0.156%; ●, 6.35%; □, 21.1%; ■, 29.2%.

were also produced as indicated by total organic carbon assays of the filtered electrolyte after extended anodic oxidation of coal.

A qualitative but sensitive mass spectrometric analysis was made of the gases produced at both anode and cathode. Note that no lines were observed for molecular weights corresponding to SO_2 or H_2S , even though the parent lignite contains significant sulphur.

We can compare the efficiency of our process (coal-consuming water electrolysis) with ordinary water electrolysis. In water electrolysis the energy required to split the water molecule is supplied solely by electricity, whereas in our new process the required energy is supplied only in part by electricity with the balance arising by way of the concomitant anodic oxidation of coal. The following quantitative development gives a first order approximation of how much energy comes from each such source and thereby provides a rough feeling for efficiency. The energy consumed by conventional water electrolysis conducted at a potential of E_2 to produce N_{H_2} moles of H_2 is $2N_{\text{H}_2}FE_2$ whereas the energy required by the present process operating at a potential of E is:

$$E \int_0^t i \, dt + N_C(-\Delta H)$$

where E is the potential applied across the cell; i is the current; and t is time N_C is the number of mol of carbon consumed and ΔH is the enthalpy of combustion of carbon to CO_2 .

The foregoing expression can be simplified by assuming a constant operating potential E , and noting that:

$$N_{\text{H}_2} = \int_0^t i \, dt / 2F \quad \text{and} \quad N_C = 1/2 N_{\text{H}_2}$$

where F , the Faraday constant, is $96,500 \text{ C equiv.}^{-1}$

Eliminating $\int_0^t i \, dt$ and N_C the expression for total energy consumption by our process becomes

$$2FN_{\text{H}_2}E + 1/2N_{\text{H}_2}(-\Delta H)$$

Received 18 December 1978; accepted 21 March 1979.

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The relative energy usage (REU) is accordingly:

$$\begin{aligned} \text{REU} &= \left(\frac{\text{ordinary electrolysis}}{\text{coal-assisted electrolysis}} \right) \\ &= \frac{2N_{\text{H}_2}FE_2}{2FN_{\text{H}_2}E + 1/2N_{\text{H}_2}|\Delta H|} \\ &= E_2/(E + |\Delta H|/4F) \end{aligned}$$

Inserting $|\Delta H| = 94,100 \times 4.18 \text{ J mol}^{-1}$ and the value of F gives:

$$\text{REU} = E_2/(E + 1.02)$$

Practical values of E_2 for conventional electrolysis are about 2 V whereas values of E observed in the present work have ranged from ~ 0.8 to ~ 1.0 V at room temperature. This means that the total energy consumption is about the same ($\text{REU} \approx 1$), per unit of hydrogen produced, for ordinary water electrolysis and for coal-assisted water electrolysis conducted in the experiments near room temperature reported here. In the case of electrochemical gasification to hydrogen, however, about half the required energy comes directly from coal and half from electricity. We expect that the total energy requirement for coal-assisted water electrolysis can be lowered further by carrying it out at higher temperatures thereby permitting operation at lower potentials (E) than 0.8–1.0 V. Note that we have treated coal as pure carbon and assumed it is completely oxidised to CO_2 at the anode in the above approximate assessment of REU. Such simplification ignores the complications arising from the variable hydrogen and oxygen content of different coals and the question of the different relative proportions of CO, CO_2 and small amounts of non-gaseous carbon oxides that may be formed at different operating conditions.

The results presented here represent a new technical concept for preparation of hydrogen from the electrochemical reaction of water and coal. Additional results will be reported elsewhere.

We thank the University of Connecticut and the US Department of Energy for financial support. Larry Veneziano provided experimental assistance.

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letters

The origin of baryons in the Universe

THERE are several clues to the history of the early Universe. The 3-K microwave background indicates that the Universe was highly isotropic when it was about a half million years old, and the abundance of ^4He (also D, ^3He and ^7Li) indicates that nucleosynthesis was taking place when the Universe was about three minutes old. These two facts are strong evidence that the Universe began from a hot big bang¹. In addition, Hawking, Ellis and Penrose have proved (within general relativity) that the existence of the 3-K radiation implies the Universe must have been singular in its past^{2,3}. The existence and clustering of galaxies indicate there were some deviations from homogeneity in the early Universe. Perhaps the most curious fact about the

Universe is that it is composed almost entirely of matter (the Universe contains negligible amounts of antimatter) and that the number of baryons per photon ($\sim$ baryon/entropy ratio) is between 10^{-10} and 10^{-8} (ref. 4). If baryon number is absolutely conserved, then it follows that the baryon number of the Universe must be viewed merely as an initial condition. Recent ideas in particle physics when applied to the early Universe may explain how an initially baryon-symmetrical (zero net baryon number) Universe could have evolved into one with net baryon number. Here, we will discuss these ideas and other interesting astrophysical implications.

The striking success of the Weinberg–Salam $\text{SU}(2) \times \text{U}(1)$ gauge theory of the weak and electromagnetic interactions⁵ and of the $\text{SU}(3)$ colour gauge theory of the strong interactions^{6,7} has

motivated grand unified gauge theories of the strong, weak and electromagnetic interactions⁸⁻¹². A common feature of all these grand unified theories is baryon non-conservation mediated by a very heavy (Higgs and/or gauge) boson(s) of mass $\sim 10^{10}$ – 10^{16} GeV c^{-2} . In addition, these theories naturally violate CP (particle-anti-particle symmetry).

At present energies (even those of the largest proposed particle accelerators), these baryon non-conserving processes are not important; however, in the early Universe, when $kT \geq m_x c^2$ (m_x is the mass of the boson which mediates baryon non-conserving reactions) these processes would have been roughly the same strength as the other interactions and an initially baryon-symmetrical Universe could have evolved into one with net baryon number. Several authors¹³⁻¹⁶ have pointed out that in addition to CP and B violations one must also have departures from thermal equilibrium to produce baryon asymmetry. Departures from equilibrium occur naturally in the early Universe when reaction rates cannot keep pace with the expansion rate of the Universe. Several schemes have been proposed^{13,14,16-19} which produce a baryon/photon ratio of $\sim 10^{-10}$ – 10^{-8} . We shall describe the one proposed by Weinberg¹⁶, and discuss the astrophysical consequences of cosmological baryon production and a new hybrid scheme using an earlier idea of Hawking²⁰ as well as the new unification theories.

In the Weinberg scheme, the Universe begins as a baryon-symmetrical hot soup containing all the fundamental particles (leptons, quarks, photons, X-bosons). When the lifetime of the X-boson is approximately the age of the Universe, kT is less than $m_x c^2$, so that the X- and $\bar{X}$ -bosons freely decay (their decay products are not energetic enough to regenerate them). At this temperature baryon non-conserving reactions have become ineffective (reaction rates < expansion rate) so that any baryon number generated will not be destroyed. The decay products of the $\bar{X}$ -bosons have a mean net baryon number $B_{\bar{X}} \neq -B_X$, B_X = the mean net baryon number of the decay products of X-bosons (this is permitted by CP and B violations). Therefore, the Universe acquires a baryon/photon ratio proportional to $(B_X + B_{\bar{X}})$, which is itself proportional to the size of the CP violation. From this point forward the Universe evolves with the baryon/photon ratio remaining constant or decreasing with the generation of additional entropy or with the decay of baryons.

Because grand unified theories do not conserve B, they also predict that the proton is unstable. In the SU(5) and O(10) unification theories, $\tau_p \sim \alpha^{-2} m_x^4 / m_p^5 \sim 10^{32 \pm 1}$ yr (α ~ fine structure constant $\approx 1/137$)^{21,22}. The current lower limit on the proton lifetime is 10^{29} yr and comes from the Case-Wit-Irvine neutrino experiment in South Africa²³. Lande *et al.* are using the neutrino telescope at Homestake Mine in Lead, South Dakota (for this experiment neutrinos are the background!) to explore lifetimes of $\sim 10^{31}$ yr (by mid-1979) and hope to be able to have developed a sensitivity to lifetimes of $\sim 10^{32}$ yr within 2 yr (K. Lande, personal communication). A positive result from a proton decay experiment would be strong evidence for baryon generation in the early Universe. In addition, if the Universe is open, such a result means that it will eventually be devoid of baryonic material.

In the gravitational instability theory of galaxy formation and clustering, small initial density fluctuations grow with time and eventually become of the order of unity, leading to the formation of galaxies and clusters of galaxies²⁴. The initial fluctuations are of two types: adiabatic and isothermal. In an adiabatic fluctuation both the matter and radiation are perturbed and are related by $(\delta\rho/\rho)_{\text{matter}} = 3(\delta T/T)_{\text{radiation}}$. In an adiabatic fluctuation the baryon/entropy ratio remains constant. In an isothermal fluctuation the radiation does not participate in the perturbation ($\delta T/T = 0$) and so the baryon/entropy ratio is not constant. The adiabatic perturbations are subject to damping (while the matter and radiation are coupled) and only fluctuations on mass scales $\geq 10^{12} M_\odot$ will survive and grow to the order of unity. Isothermal perturbations are not subject to damping, and fluctuations on mass scales $\geq 10^5 M_\odot$ will grow to the order of unity. If the initial fluctuations were adiabatic then the 3-K background should

show variations $\Delta T/T \sim 10^{-3}$ on angular scales of ~ 30 arc s. Such variations have not been seen and are just below the sensitivity level of current experiments²⁵.

If the baryons in the Universe were generated as outlined above, the baryon/entropy ratio would depend only on micro-physics (the grand unified theory). Therefore, the initial temperature fluctuations in the Universe would not give rise to baryon/entropy fluctuations (unless the fluctuations were so large that some parts of the Universe were never hotter than $m_x c^2/k$ and no net baryon number was generated). Any initial density perturbations must be adiabatic rather than isothermal. This fact is related to the problem of galaxy formation and clustering in the Universe.

One way around the elimination of initial isothermal fluctuations is through primordial black holes. Hawking has shown that a black hole of mass m (in grammes) should emit a spectrum of particles like a black body with a temperature of $\sim 10^{26} K m^{-1}$ (ref. 26). As a black hole radiates, it loses mass and ultimately evaporates in a time $\sim 10^{-26} m^3 N^{-1} s$ (N ~ number of particle species it can emit)²⁷. As it is evaporating its temperature increases; when $kT \sim m_x c^2$ a black hole can emit X- and $\bar{X}$ -bosons (in equal numbers). When these bosons decay they will produce a net baryon number $\sim (B_X + B_{\bar{X}}) \times (\text{number of X, } \bar{X}\text{-pairs emitted})$. All black holes with an initial mass m such that the corresponding temperature is $\leq m_x c^2/k$ ($m \geq 10^{13} m_x^{-1}$, m_x in GeV) will eventually produce the same number of X, $\bar{X}$ -pairs and hence the same net baryon number. (This is because no X, $\bar{X}$ -pairs will be produced until the mass of the black hole has decreased such that its temperature $\sim m_x c^2/k$.) The rest of the mass of an evaporating black hole will eventually be converted into photons (entropy), so that the baryon/entropy ratio created by an evaporating black hole is proportional to m^{-1} . The entropy and baryon number of the Universe could be produced from an appropriate primordial spectrum of black holes (Hawking first suggested baryon and entropy production by primordial black holes, although he did not elaborate on a mechanism²⁰). In addition, as the baryon/entropy ratio produced by an evaporating black hole depends on its mass, the distribution and mass spectrum of primordial black holes could lead to both isothermal and adiabatic initial density fluctuations in the Universe. For example, suppose the Universe was initially isothermal and without net baryon number. Let the net baryon number be produced by a non-uniform spatial distribution of primordial black holes of mass m . If the entropy added by the evaporation of the black holes is small (let m be $\sim 10^{13} m_x^{-1}$ g) the density fluctuations caused by the spatial distribution of black holes will be nearly isothermal. For some values of m_x , baryon production by other routes is negligible because when the X and $\bar{X}$ decay baryon non-conserving collisions are still effective and any baryon excess produced is neutralised. In these cases primordial black holes can act as 'seeds', evaporating after nm-conserving collisions become ineffective and thereby generating a baryon excess that is not neutralised.

In conclusion, we can say that recent developments in the unification of elementary particle interactions by gauge theories may provide an explanation for what seemed to be an arbitrary initial condition—the baryon/entropy ratio. In addition, if this explanation is correct, it is directly relevant to the question of initial density fluctuations and galaxy formation.

We thank S. Weinberg, G. Steigman, W. Press and D. Nanopoulos for helpful conversations. This work was supported in part by NSF grant AST 78-20402 and an Enrico Fermi Fellowship at The University of Chicago to M.S.T.

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Received 8 February; accepted 30 March 1979

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IUE observations of the symbiotic star CH Cygni during an active phase

THE spectrum of CH Cygni usually has the appearance of a normal M6 III star, but at intervals of several years, it has outbursts during which it shows the characteristics of a symbiotic star, that is a blue continuum extending shortward of the Balmer discontinuity, and emission lines of He I, Fe II, [Fe II], [S II], [O III]; the Balmer lines and the H and K lines of Ca II present emission wings. The observations of CH Cygni reported here were made to determine whether a symbiotic star is a binary system composed of an M6 giant and a hot subdwarf, or whether it is a cool star surrounded by a thick corona.

Outbursts were observed: in September 1963 (ref. 1), the star had already returned to normal by August 1965; in June 1967 (ref. 2) the spectrum was followed by Faraggiana and Hack³ until December 1970 when the star returned to normal; another

outburst was observed in August 1977 (refs 4, 5), the spectrum was observed by Faraggiana with the coude spectrograph at the 152-cm telescope of the Haute Provence Observatory, and in June and September 1978 still had the characteristics of a symbiotic star. Our observations of the UV spectrum of CH Cygni reported here, made with IUE on April and July 1978 were therefore taken during its active phase.

The most obvious hypothesis for explaining symbiotic stars is that they are binary systems formed of a late-type giant and a hot subdwarf which occasionally has outbursts. Or the cool star may be surrounded by an extended and thick corona heated by shock waves, which is supposed to be responsible for the blue continuum and the emission lines^{6,7}. A third hypothesis⁸, that a hot star is surrounded by a cool, optically thick envelope, was discarded by the observations of CH Cygni in 1967–70 (ref. 3) because they gave clear evidence that the blue continuum was filling up the M6 photospheric lines, a distinctive phenomenon which was again observed in the spectra obtained by Faraggiana in June and September 1978 (personal communication).

Several symbiotic stars present orbital motions; however, CH Cygni does not show any evidence of periodic radial velocity variations. Instead, our observations supported the hypothesis that shock waves heat the extended envelope surrounding the M6 giant star. Photometric observations by Slovak and Africano⁹, however, indicate that CH Cygni presents a rapid flickering, characteristic of the cataclysmic variables, most of which are close binary systems. According to Slovak and Africano⁹, the flickering provides the strongest evidence for the interpretation of CH Cygni as a binary system undergoing mass transfer.

To decide which hypothesis could explain the spectrum of CH Cygni, it was observed with IUE, in the low resolution mode and in the short-wave range on 22 April (using the 3" aperture) and on 31 July 1978 (using the large aperture, 10"×20").

The UV spectrum of CH Cygni is characterised by an almost flat continuum from $\lambda 1250$ to $\lambda 1700$ with a mean flux of about 5×10^{-13} erg s⁻¹ cm⁻² Å⁻¹ (determined from the July spectrum, observed with the large aperture), by a very strong emission at $\lambda 1302$ (the flux at the centre of the emission is about 5 and 4 times the flux in the adjacent continuum, in April and July respectively) due to O I, and by several fainter emissions, which in many cases can be barely distinguished from high points on the continuum; moreover, several absorption lines are present. The energy distribution in the continuum at $\lambda > 1700$ is strongly

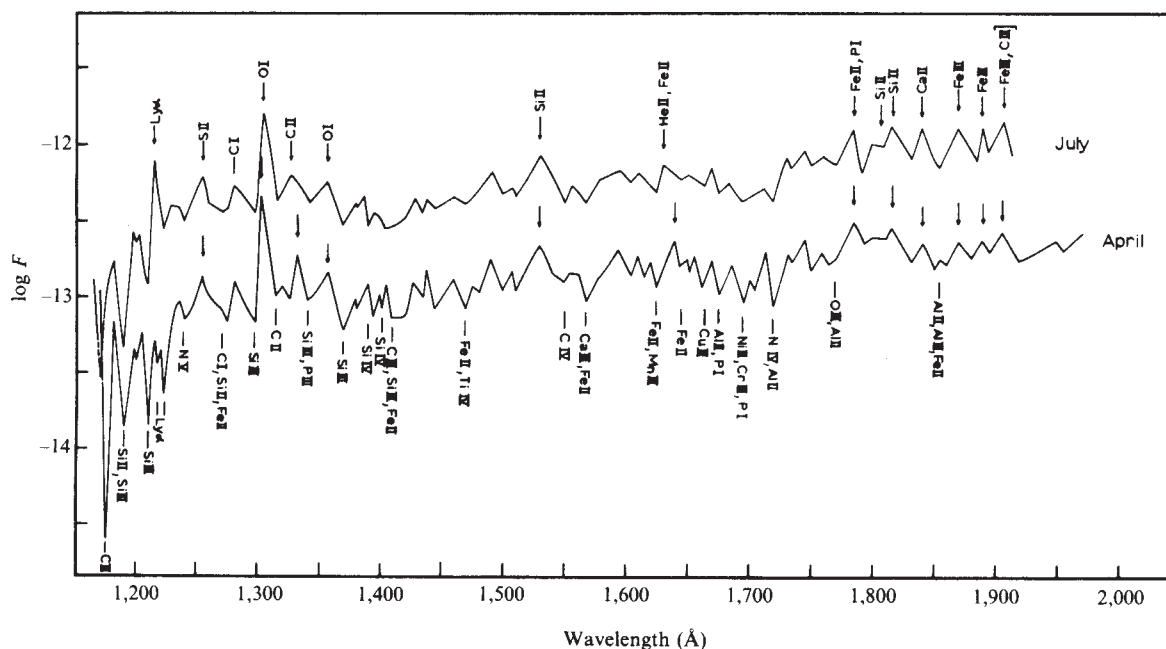


Fig. 1 The spectra of CH Cygni observed in April and July. The ordinates are $\log F$, where F is the flux at the Earth (erg cm⁻² s⁻¹ Å⁻¹) given by the relation $F_{\lambda} = N_{\lambda} S_{\lambda}^{-1} t^{-1}$, where N_{λ} is the IUE flux number, S_{λ} the camera sensitivity and t the exposure time in seconds. The emissions are indicated by arrows.

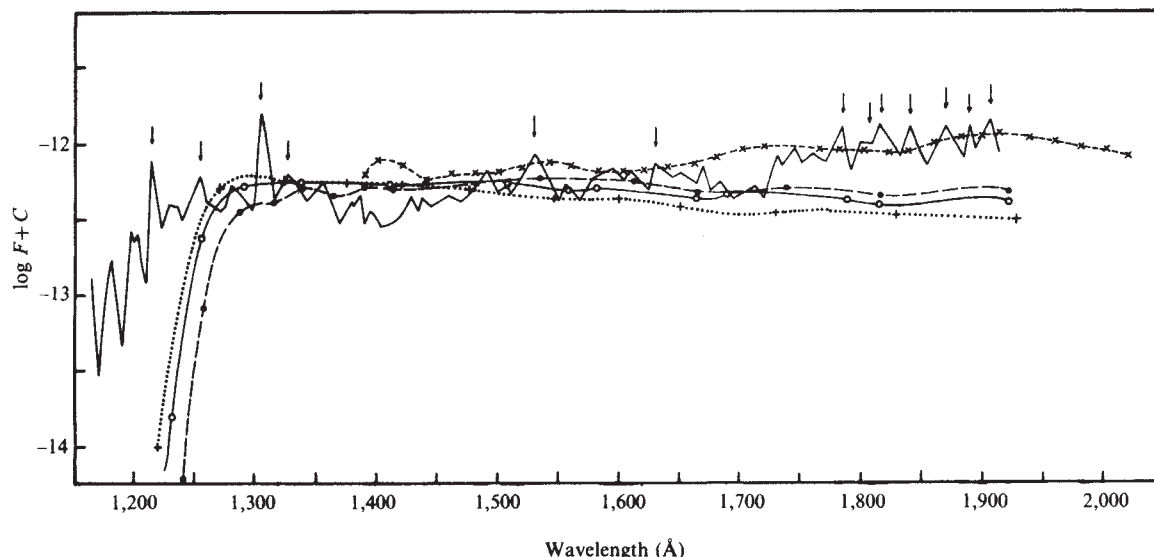


Fig. 2 The far UV spectrum of CH Cygni, observed on 31 July 1978, is compared with that of Beta Lyrae (S2/68 observations) and with the Kurucz, Peytremann and Avrett blanketed models. $\times$ — $\times$, Beta Lyrae; $\bullet$ — $\bullet$, KPA model, $T_e = 10,000$ K, $\log g = 4.5$; $\circ$ — $\circ$, $T_e = 11,000$ K, $\log g = 4.5$; $+$ $\cdots$ $+$, $T_e = 13,000$ K, $\log g = 4.5$. The arrows above the CH Cygni spectrum indicate the position of the emission lines.

affected by the presence of the emissions. Their blending produces in this region a peculiar energy distribution, very similar to that previously observed for Beta Lyrae¹⁰ with experiment S2/68 (spectral resolution about 35 Å) aboard the European satellite TD-1.

The two spectra observed in April and July are almost identical, except for a constant difference in the flux, which is four times lower in April, a difference probably due exclusively to the light loss through the 3" aperture¹¹ (Fig. 1). Moreover, the July spectrum presents a strong emission at Ly α , which is of geocoronal origin, because it fills up the whole height of the slit, as shown by inspection of the photowrite. No appreciable variations are evident, either, in the region $\lambda\lambda 3500$ – 5000 Å, at least from a visual inspection of the spectra taken in June and September 1978.

As it is very difficult to trace a reliable continuum through the various absorption and emission features, we have considered an average curve passing through the majority of these features, but below the strongest emissions and above the strongest absorptions (Fig. 2). We then compared this with the spectra of several stars observed with S2/68 (ref. 12), and with several theoretical models¹³. The best fit along the whole region 1380–1900, common to IUE and S2/68 observations, is obtained with Beta Lyrae, whose spectrum is also characterised by the presence of several strong emissions. As Beta Lyrae has been observed in the high resolution mode with IUE, the identification of the emissions in its spectrum has been a useful guide to the identification of the emissions in the spectrum of CH Cygni. Comparison with normal unreddened stars indicates that the best agreement is found with B9–A0 stars, but only in the region $\lambda < 1750$ Å, because of the distortion due to the emissions. Atmospheric models for $T_e \approx 10,000$ K or $11,000$ K agree with the observed energy distribution in the region $\lambda\lambda 1275$ – 1750 Å (Fig. 2). At $\lambda > 1750$ Å, the continuum is distorted by the blending of several strong emission features, and at $\lambda < 1,275$ Å the flux decrease is slower than that predicted by the models. This latter behaviour indicates that the Ly α absorption is much fainter in CH Cygni, an effect we attributed to the presence of geocoronal and stellar Ly α emission.

The magnitude difference Δm with the standard star HD168905, $B_2.5$ V, $V = 5.24$, observed with IUE with the large aperture (Selvelli, personal communication), ranges from +9.5 at $\lambda 1170$ to +6.0 at $\lambda 1900$. The magnitude difference with Vega (observed with S2/68 (ref. 12) ranges from +10.3 at $\lambda 1450$ to +9.3 at $\lambda 1900$.

The main contributors to the blends are given in Fig. 1. Absorption lines of multi-ionised atoms like $\lambda 1175$ C III, $\lambda 1550$ C IV, $\lambda 1718$ N IV, $\lambda 1240$ N V, probably $\lambda 1767$ and $\lambda 1771$ O III, $\lambda 1400$ Si IV are present. All the emission lines, on the contrary, are due to transitions to the ground level of neutral or once-ionised atoms (like $\lambda 1253$ S II, multiplet 1 (ref. 14); $\lambda 1281$ C II, 5; $\lambda 1303$ and $\lambda 1355$ O I, 2; $\lambda 1335$ C II, 1; $\lambda 1533$ Si II, 2; $\lambda 1785$ P I, 1; $\lambda 1808$ Si II, 1; $\lambda 1817$ Si II, 1) and to the strongest lines of iron twice-ionised. The emission at $\lambda 1640$, attributed to He II, probably receives an important contribution from the Fe II lines of multiplets 8 and 68, which have the second strongest intensities in this spectral region, after those of multiplet 191, which is a possible explanation for the emission at $\lambda 1785$, together with P I.

These observations do not enable us to decide between the two hypotheses outlined above. The UV continuum seems to be the continuation of the optical violet continuum observed during outbursts, whose colour temperatures was found to equal $\sim 10,000$ K (ref. 3). This temperature is not sufficient to explain the presence of the absorption lines of multi-ionised atoms. If we assume that the UV continuum is due to an increase in brightness of the hypothetic companion or that it arises in a hot corona surrounding the cool star and which becomes optically thick during the active phases then we must assume that mechanical energy, liberated during the outburst, is responsible for the high degree of ionisation of the gas. In contrast the emission lines should form in an extended envelope. The presence of this envelope, where hydrogen is mostly ionised, is indicated by the strong emission of O I at $\lambda 1302$. In fact Ly β and $\lambda 1025.77$ O I have almost the same high excitation potential: therefore, Ly β emission overpopulates the upper level of $\lambda 1025$ O I; hence by cascade, emissions at $\lambda 11287$, $\lambda 8446$ and $\lambda 1302$ are explained¹⁵.

It would be necessary to observe the UV spectrum of CH Cygni in periods of quiescence to understand better the nature of the companion, if present, and to study the conditions of the envelope far from the effects of the outbursts.

Note that CH Cygni has been observed again with IUE on 11 March 1979 in the low resolution mode. The spectrum is identical with that observed on July 1978, but the flux is increased by a factor 1.6 over the whole spectral range 1,200–1,900 Å.

This research is based on observations by the IUE collected at the Villafranca Satellite Tracking Station of the ESA. I thank all the staff of the Villafranca Station for their assistance in observ-

ing and reducing the data, and especially Dr P. L. Selvelli for the July observations. This work was supported by a CNR-SAS contract.

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Received 22 January; accepted 2 April 1979.

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Temperature structure of the uranian upper atmosphere

FROM observations of the occultation of the star SAO158687 by Uranus on 10 March 1977^{1,2}, we have determined the temperature structure of its upper atmosphere at two locations on the planet. These are the first temperature measurements within the 10^{-4} – 10^{-2} mbar range, which lies several scale heights above the region probed by IR measurements. Although this occultation was noted for the unexpected discovery of the uranian rings^{3–5} and ring occultations were observed by several groups⁶, an occultation by the planet itself was seen from only two observatories—the Kuiper Airborne Observatory (KAO) and Cape Town. Here we present the first results from the KAO data: the Cape Town results will be discussed elsewhere⁷.

The data we obtained with the 91-cm telescope aboard the KAO consist of continuous records of the starlight intensity, recorded simultaneously at three wavelengths (6,190; 7,280 and 8,520 Å) with a time resolution of 10 ms. These wavelengths, which correspond to methane absorption bands in the spectrum of Uranus, were chosen to enhance the signal-to-noise ratio of the data by discriminating against the background light from Uranus². The immersion and emersion records contained many 'spikes' as were observed during occultations by Jupiter and Neptune⁸. A variable component of scattered moonlight, amounting to a few per cent of the unocculted stellar intensity, was removed from the light curves before analysis. Details of our observations are given elsewhere^{3,9}.

The light curves were analysed by the inversion method of French *et al.*¹⁰ to yield the number density, $n(h)$, pressure, $p(h)$, and scale height, $H(h)$, of the uranian atmosphere as a function of height h in the atmosphere. [Note that inversion of occultation light requires the validity of certain general assumptions; see refs 11–16.] The temperature $T(h)$ was determined through the relation $T(h) = \mu g H(h)/R$, where μ is the mean molecular weight of the atmosphere, g is the local gravitational acceleration and R is the universal gas constant. Here we have set $g = 830 \text{ cm s}^{-2}$, which corresponds to a rotation period of 24 h, and $\mu = 2.2 \text{ g mol}^{-1}$, which corresponds to an atmosphere of hydrogen and helium in their solar abundances. At these altitudes the concentration of methane would be much too low to significantly affect the mean molecular weight¹⁷.

The temperature–pressure relationships obtained from the immersion and emersion data for our 7,280 Å channel are

shown in Fig. 1. The shaded region in the upper part of each figure represents the initial temperature and its uncertainty obtained from the initial conditions that were used to begin the numerical inversion of the data. The temperature profiles terminate at high pressures (lower altitudes) when the light flux has become small enough for the uncertainty in the zero flux level to produce a significant error in the profiles. The error bars on the profiles are due to the effects of noise in the original light curves. The scatter of adjacent points is less than the length of the error bars because the photon noise in the light curve produces correlated errors in the profiles, as discussed by French and Elliot¹⁸.

The temperature profiles in Fig. 1 show peak-to-peak variations of 45 K for immersion and 35 K for emersion. The temperature variations at higher altitudes are probably an artefact of the inversion procedure¹⁸, so that the actual temperature variations may be somewhat less than shown in Fig. 1 (particularly for emersion). Hence the peak-to-peak variations of

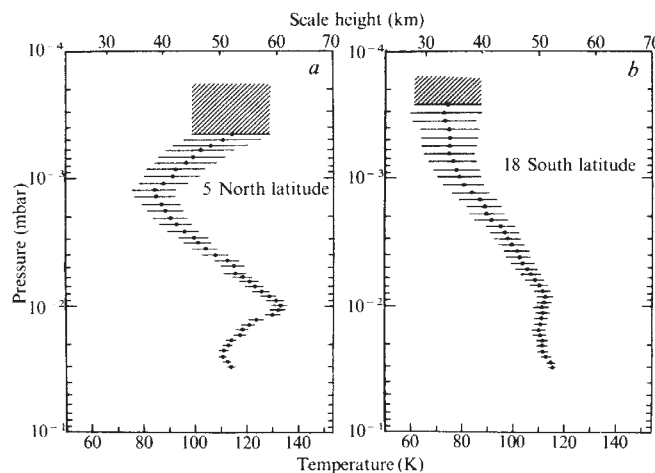


Fig. 1 Temperature–pressure relationships for the upper atmosphere of Uranus. The temperature of the upper atmosphere of Uranus is plotted against pressure for *a*, the immersion data and *b*, the emersion data. The mean temperature of each profile is about 100 K, which shows that Uranus has a temperature inversion between 10^{-3} mbar and the 10^{-2} mbar level probed by IR measurements, where the temperature is 58.5 K. Both profiles show wave-like temperature variations, which may be due to dynamical or photochemical processes. Nearby points differ by less than the calculated error bars because the photon noise in the original data produces correlated errors in the temperature–pressure relationships. (Data from KAO Ch. 2).

35–45 K must be considered as maximum values. However, we believe that a large component of these variations is real and indicates that the atmosphere of Uranus is not isothermal in this region for three reasons: (1) the same variations occur in the temperature profiles derived from all three of our photometric channels; (2) the variations are much larger than the error bars, indicating that they cannot be attributed to photon noise in the data; and (3) similar variations occur in the profiles obtained from the Cape Town data. The variations may be due to dynamical processes in the atmosphere or photochemical layering, as discussed below.

The temperature gradients are sub-adiabatic everywhere, although the gradient near $p = 10^{-3}$ mbar is very close to the dry adiabatic gradient. The condensation boundaries for CH_4 , NH_3 and H_2O all lie well to the left of the profile, if we assume that the ratio of the number densities of these molecules to the hydrogen number density is fixed at the saturation value of the temperature minimum at the 100-mbar pressure level.

The mean temperatures of the region probed by the occultation events, obtained by averaging the points in each profile,

are 109 K for immersion and 96 K for emersion. We also obtained mean temperatures of 122 K and 110 K by fitting a model occultation curve¹⁹ for an isothermal atmosphere to the data, but prefer the mean temperatures obtained from the inversion profiles since the atmosphere is definitely not isothermal in this region. The higher temperature obtained from the immersion data may be due to the 'phase' of the temperature variations and not represent an actual difference between the mean temperatures that would be obtained for these two regions by averaging over a sufficiently long time or a larger altitude range. We note that immersion occurred just before sunrise at latitude 4.5° north and emersion occurred just after sunrise at latitude 18.1° south. The subsolar latitude at the time of the occultation was 51.0° north, and the phase angle was 2.4°.

Summarising our conclusions about the thermal structure of Uranus in the 10^{-4} – 10^{-2} mbar region: (1) the mean temperature is about 100 K, (2) the atmosphere is not isothermal and (3) the temperature variations are larger in the region sampled by immersion than that sampled by the emersion event.

The mean temperature of 100 K at the occultation level is about 40 K higher than the IR temperature, implying that a temperature inversion occurs between the 10^2 and 10^{-3} mbar levels. The cause of the inversion may be heating of the upper atmosphere by methane, as postulated for the models of Wallace²⁰, however, his model temperatures are warmer than the temperatures found here. His 'saturated' model is in better agreement than his 'supersaturated' model. Another possible explanation for the temperature inversion is the dissipation of energy by dynamical processes, if these are the cause of the observed temperature variations. However, the problem with this mechanism is that the possible dynamical processes identified so far would heat the atmosphere to a temperature well above the observed value²¹.

Table 1 Temperatures of Uranus and Neptune

	Uranus	Neptune
Mean distance from Sun	19.2 AU	30.1 AU
Effective temperature (IR, $p \sim 10^2$ mbar)	58.5 ± 2 K (ref. 22)	59.7 ± 4 K (ref. 22)
Occultation temperature ($p \sim 10^{-3}$ mbar)	100 K	140 K (refs 25–27)
Heating mechanism at $p \sim 10^{-3}$ mbar	CH ₄ (?) (ref. 20)	CH ₄ (?) (ref. 20)
Temperature variations (peak-to-peak) at $p \sim 10^{-3}$ mbar	35–45 K*	5–25 K (refs 25, 26)*
Origin of temperature variations	? (ref. 21)	? (ref. 21)

* Maximum value, see text for discussion

The thermal structure of Uranus is somewhat different from that of Neptune, as is shown by the summary of IR and occultation temperatures in Table 1. Both planets have nearly the same effective temperatures, and this has been interpreted as showing that Neptune radiates about 2.8 times the energy it receives for the Sun, while Uranus radiates no more than 1.23 times the energy than it receives^{22,23}. If Uranus has any internal heat source at all, it must be a smaller fraction of the solar energy received than the internal heat sources of the other giant planets^{23,24}. The temperature inversion between the regions probed by IR and occultation measurements is much greater for Neptune than for Uranus. This is consistent with the conclusions of Gillett and Rieke¹⁷, who found a stronger temperature inversion for Neptune on the basis of methane and ethane emission at 8 and 12 μ m.

Both planets show temperature variations in the region probed by occultations. However, the cause of the variable temperatures and their relationship to radiative processes occurring in the upper atmospheres of these planets remain unknown.

We thank C. M. Gillespie, Jr and the crew of the Kuiper Airborne Observatory for their assistance and cooperation, and

P. J. Gierasch and R. G. French for discussions. This work was supported in part by grants from NASA and NSF.

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Electric currents in power transmission line induced by auroral activity

SURGES of electric currents induced by auroral activity were first observed in the middle of the last century in the wires of the electric telegraph^{1,2}. In more recent years, auroral effects on long conductors, such as electric power transmission systems, telephone cables and oil pipelines, have attracted much attention because of various problems caused by these currents³. We report here measurements of the currents in a local power transmission line between Healy and Fairbanks and show that some of the fluctuations are aurorally induced.

The electric currents (induced by auroral activity) are caused by transient geomagnetic field variations which arise from concentrated ionospheric currents, called the auroral electrojets. Since the Earth is a spherical conductor, during intense auroral activity transient magnetic variations caused by the growth and decay or a rapid movement of the electrojet produce an induced Earth-surface potential. The induced potential in turn produces currents in a conductor, such as power, communication or pipeline systems, that are grounded to the Earth at two separate points^{4,5}.

This induction effect is greatly complicated by various factors, such as the geometry of the conductor system with respect to the auroral electrojet and the geological structure in the vicinity of the conductors⁶. These effects are difficult to quantify in modelling the induction effect. Thus, to understand effects of the induced currents on powerline systems, the magnitude of the current must first be determined experimentally and the relationship between characteristics of geomagnetic variations and the induced currents in power line systems examined. However, no previous report has demonstrated clearly that some of the disturbances in power transmission lines are caused by auroral activity on the basis of simultaneous power line records and auroral activity records.

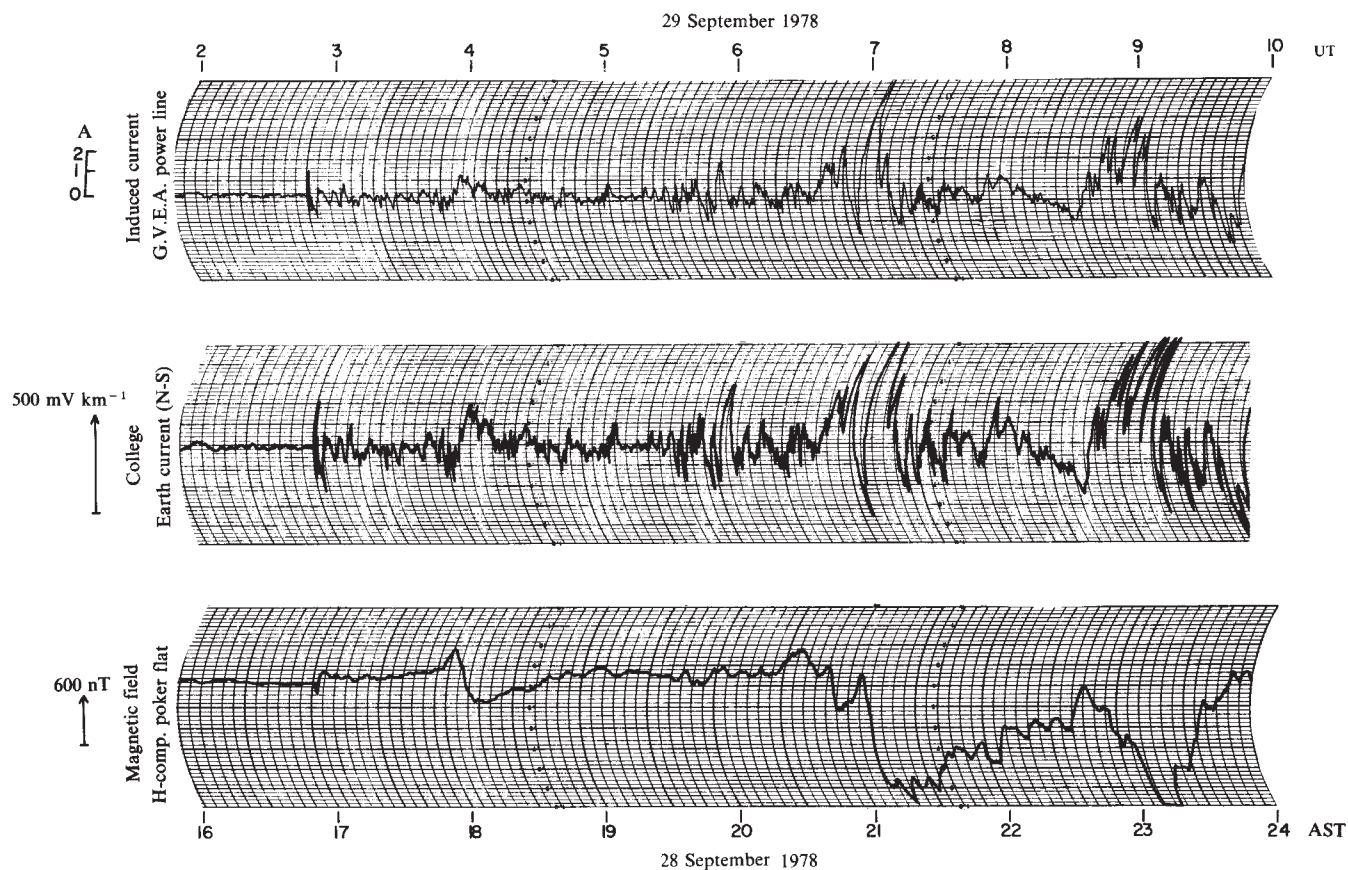


Fig. 1 Comparison of the induced current in the Healy–Fairbanks power line transmission with the north–south component of the Earth current monitored in the same vicinity, and the **H** component magnetic records; 02.10 UT, 29 September, 1978 (16.24 AST, 28 September, 1978); AST, Alaska standard time.

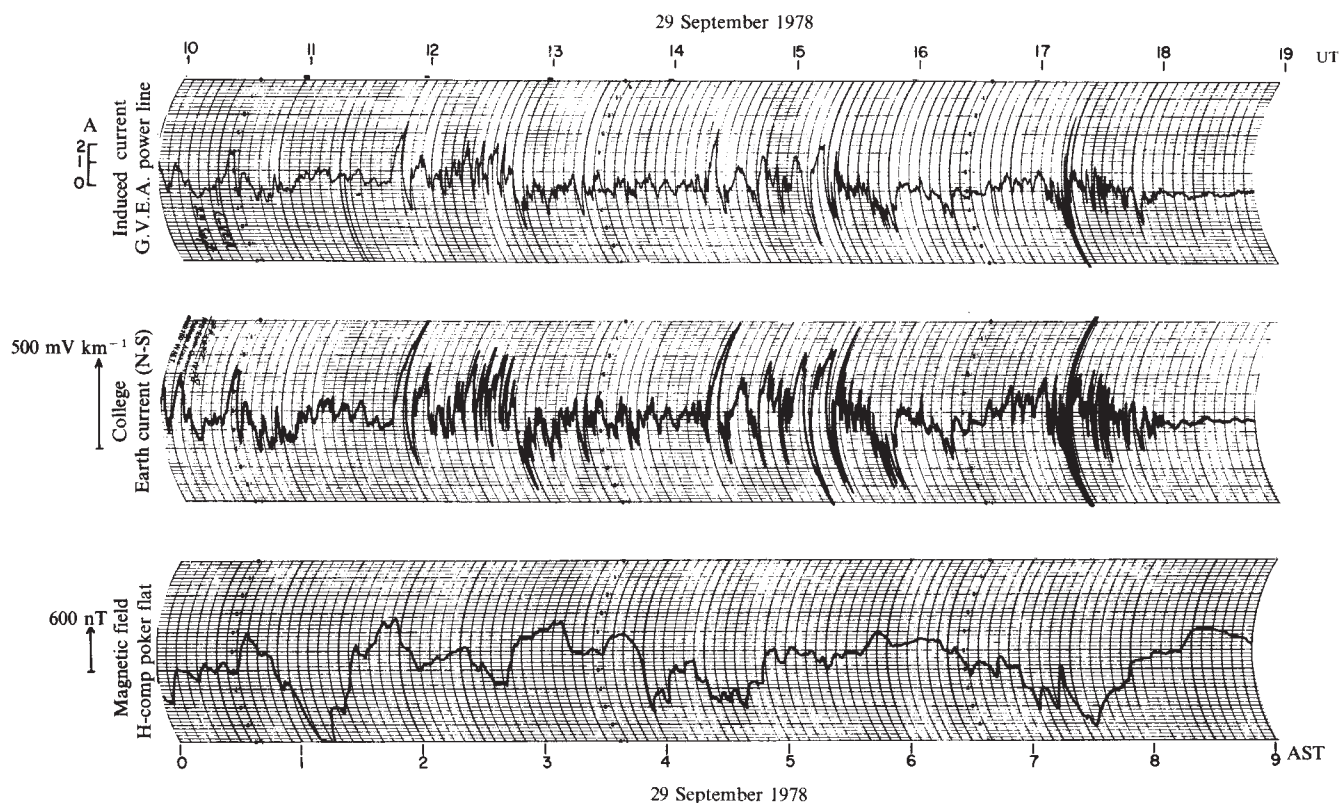


Fig. 2 As for Fig. 1; 10.19 UT, 29 September, 1978 (0.9 AST, 29 September, 1978).

We have therefore begun to monitor the induced current in a local power transmission line (138 kV, 100 A) in the vicinity of Fairbanks, Alaska, along a grounded neutral transmission line between Healy (the generator site) and Fairbanks, of length of 166 km.

Figures 1 and 2 show both the induced current in the Healy-Fairbanks transmission line at one of the transformer sites (substations) in Fairbanks. We show here the simultaneous **H** component magnetic record and the induced electric field (commonly referred to as 'the earth or telluric current') record taken in Fairbanks as indicators of auroral activity and the auroral electrojet. By comparing the induced current in the Healy-Fairbanks transmission line and the Earth current record it is obvious that the fluctuating currents in the transmission line were induced by the auroral induction. The corresponding negative excursions in the **H** component magnetic record indicate that auroral substorms were in progress during the intense indication events (see ref. 7 and note also that the induction at 03 UT was caused by the sudden commencement of the storm of 29-30 September, 1978).

In the Fairbanks area, multiple ground points exist, making the measurement of the total induced current difficult. By developing a model of the grounding network, however, we have been able to determine approximately the total current flowing in the Healy-Fairbanks line from our measurement at the substation. On the basis of our measurement and the model study, it is indicated that excursions of ± 4 A recorded at the substation would represent ± 8 A d.c. flowing in the transmission line.

The voltage and current induced in a transmission line are proportional to its length. In spite of the severity of magnetic disturbances in the auroral zone, the Healy-Fairbanks transmission line has escaped major troubles from the induction effects in the past because of its relatively short length. A transmission line across the auroral zone of length more than 500 km will require a careful design to overcome the difficulties caused by the induced currents.

We thank the personnel of Golden Valley Electric Association, Fairbanks, Alaska, for their cooperation in this study. The results reported here were supported by the ERDA Contract AT(45-1)2096, Task 5.

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Analysis for ^{15}N by proton scattering

STABLE isotopes have a great potential as tracers in the life sciences and environmental research, but their uses are still limited compared with those of radioisotopes. Radioisotopes have been widely used as trace elements, although there are problems in handling radioactive materials and preventing environmental contamination. The increasing concern about exposure to small amounts of radioactivity has caused us to consider the advantages of stable isotopes. The detection of stable isotopes, however, is not as sensitive as that for radioactive tracers and the preparation of a sample for use in stable

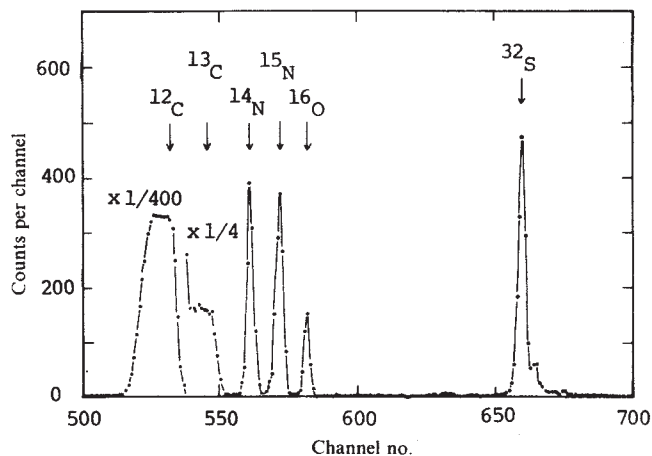


Fig. 1 Typical spectrum of protons scattered by ^{15}N in methionine-enriched 95% in ^{15}N . ^{15}N -methionine was suspended in the 1/20-diluted *Physarum* cell to 5 mg ml^{-1} . The target backing in a diameter of 12 mm was coated with $5\text{ }\mu\text{l}$ of the specimen. The target was bombarded at 6.70 MeV with an exposure of $37.9\text{ }\mu\text{C}$.

isotope analysis is complicated. We have looked at the possibility of using a nuclear reaction caused by accelerated charged particles as a way of detecting stable isotopes. We have developed a system of stable isotope analysis using proton elastic scattering. Elastic scattering was chosen because the cross-sections are generally an order of magnitude larger than that of other reactions. Moreover, proton elastic scattering analysis has many advantages which are absolute, multi-element, rapid and subject to automatic data handling. Using this method an analysis for ^{15}N has been done for biological studies. Nitrogen is an important component of various organic compounds which occur naturally but it has no long-lived radioactive isotopes. Here we outline the new system, and give an example of ^{15}N analysis.

A system for ^{15}N -labelled compounds has been developed using proton beams of the Tandem accelerator at the Research Center for Nuclear Science and Technology, the University of Tokyo. A clear peak of ^{15}N in the energy dispersion of elastically scattered protons must be obtained for even a small quantity of the isotope. For this, an optimum experimental condition of incident energy, scattered angle, beam collimation, solid angle subtended by detector and pulse height analysis system, especially, pile-up rejection system must be found. Incident proton energies were selected on the basis of the excitation functions^{1,2} of $^{15}\text{N}(p, p)^{15}\text{N}$ and of $^{16}\text{O}(p, p)^{16}\text{O}$. Because ^{16}O concentrations in natural biological samples are three orders of magnitude higher than that of ^{15}N , the peak of the elastically scattered protons by ^{16}O is too high and the tail of the ^{16}O peak masks the peak of ^{15}N . Thus, incident energies with low cross-sections for $^{16}\text{O}(p, p)^{16}\text{O}$ have been selected using the excitation function data². In addition, $^{15}\text{N}(p, p)^{15}\text{N}$ excitation function was also compared to avoid undesirably low yield of the proton by ^{15}N . We have therefore chosen several incident energies, 5.30, 5.82, 6.00, 6.30, 6.40 and 6.70 MeV, and have obtained spectra with test samples as described in the legend to Fig. 1. The incident energies of 5.82 and 6.70 MeV were selected because of a higher yield of the protons from ^{15}N at 5.82 MeV and because of the clear separation of ^{15}N peak from ^{16}O at 6.70 MeV.

We used a scattering chamber with a Si solid-state detector as the detection system for elastic scattering protons. The detector was placed 191 mm away from the target and at 165° to the beam direction. Although an angle of 180° would be desirable for sufficient energy separation to resolve ^{15}N peak from ^{16}O peak, a scattering angle of 165° was selected because of geometrical limitation. The defining slit of the detector was 9 mm in diameter and the solid angle subtended by the detector was $1.74 \times 10^{-3}\text{ sr}$. To prevent background counts due to an

accumulation of proton pulses from ^{12}C or ^{13}C involved in backing materials, a 'pile-up inspector' was used. The scattered proton spectrum was recorded using a pulse height analyser. Backing material should not contain any element heavier than N, since protons scattered by heavier elements could easily result in increased background counts in lighter mass region. Thus, carbon foil, polyethylene and polypropylene films are chosen because of the thickness of available films and their constituent elements of carbon and hydrogen. Polyethylene films were normally used because they are thin (10 μm thick), commercially available at a low cost, and samples can be easily spread on them.

Plasmodium of *Physarum polycephalum* were used for testing as it was being incubated in our laboratory³. Microplasmodia were incubated with semi-defined or synthetic medium⁴. The microplasmodia were collected by centrifugation, washed twice in water and diluted with water to a concentration of 1/20–1/10. The microplasmodia were then sonicated with an ultrasonic sonicator. The sonicated sample, volume 5–20 μl , was dropped onto the backing by pipette, spread homogeneously on the backing material and dried.

Standard samples were prepared to compare yield ratios for the proton elastically scattered by ^{15}N with respect to given concentrations of ^{15}N -methionine. DL-methionine, enriched to 95% in ^{15}N , was used. The ^{15}N -methionine was suspended in sonicated microplasmodia which were prepared from culture on nutrient medium, and spread on polyethylene backings. Suspension in the sonicated microplasmodia was necessary because the methionine suspended in water could not be spread homogeneously onto the backing.

A typical spectrum is shown in Fig. 1. The peak of ^{15}N was clearly separated from that of ^{16}O or of ^{14}N . The peak of ^{32}S appeared clearly, which consisted of protons mainly from those in the methionine added. The spectra of ^{12}C and ^{13}C were dominated by protons from those isotopes in the polyethylene backing. The peak of ^{14}N was due to protons mainly from ^{14}N in crude lysate of the cell. The ^{14}N counts were used as normalisation factor after the correction due to the contribution from ^{14}N in the added methionine. Counting ratios of $^{15}\text{N}/^{14}\text{N}$ are plotted as a function of ^{15}N concentrations in Fig. 2. A linear relationship holds between ^{15}N yields and added ^{15}N concentrations.

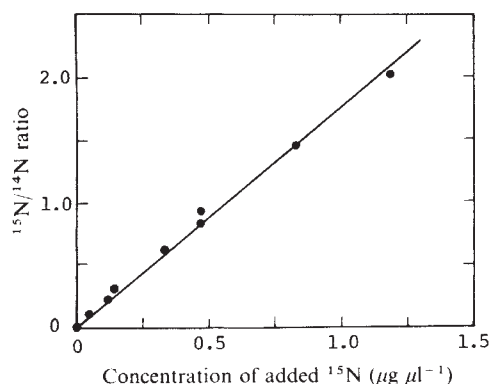


Fig. 2 A linear relationship between proton yields and ^{15}N concentrations.

An example of a proton spectrum for a sample containing ^{15}N -labelled compound is shown in Fig. 3. The sample was prepared from microplasmodia of *Physarum*, which had been incubated in the defined medium⁴ supplemented with ^{15}N -methionine (95% in ^{15}N). Twice washed microplasmodia were sonicated and spread onto the polyethylene backing. The specimen was bombarded at 6.70 MeV with a total exposure of 59 μC . The separated peaks of ^{14}N , ^{15}N and ^{16}O appeared clearly. Peaks corresponding to $^{23}\text{Na} + ^{24}\text{Mg}$, $^{31}\text{P} + ^{32}\text{S}$, $^{35,37}\text{Cl}$ and $^{39}\text{K} + ^{40}\text{Ca}$ are also discernible.

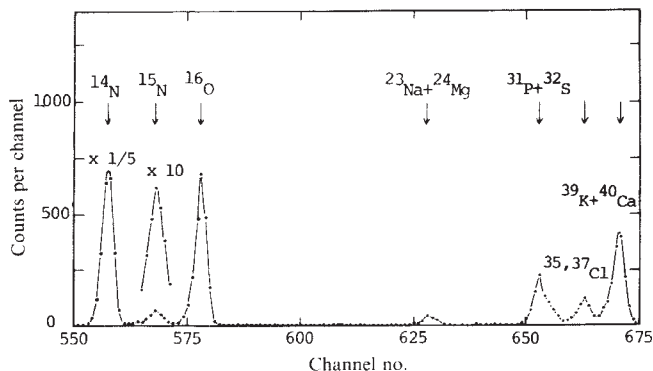


Fig. 3 Typical spectrum of protons by a bombardment of *Physarum* cell incubated with ^{15}N -methionine.

The present method has advantages in that it can easily compare the abundance ratio of several elements and easily prepare samples. Even with a natural concentration of ^{15}N , a peak of ^{15}N was discernible and analyses for such samples are thought to be possible. However, to save time the solid angle of the detector must be increased. Further work is in progress to improve the system and to apply the system to the ^{18}O analysis of biological samples.

We thank Dr Y. Yano for the preparation of the carbon foils.

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Evidence for the presence of Freon 21 in the atmosphere

MONITORING of trace constituents of the atmosphere is becoming increasingly important because of the implications on the quality of life of growing concentrations of several compounds which, after industrial use, are released to the atmosphere. In particular, concentrations of Freons and other halocarbons need to be checked because of the problem of ozone depletion^{1–4}. Recently, the search for tropospheric sinks of Freons has been considered in connection with the controversy about withdrawing such compounds from the market. The hypothesis that F21 (CHCl_2F), which has no industrial use and apparently is only produced as an impurity of F12 (CCl_2F_2), could be a decomposition product of F11, has been made by several workers^{5,6}. However, detection and quantitative analysis of this compound in the atmosphere is extremely difficult because: (1) it is present in extremely low concentration⁷; (2) gas

chromatography (GC) separation from other halocarbons is difficult⁸; (3) there are several interferences and sample contamination may easily occur. The presence of F21 in the atmosphere, although detected in several researches, is therefore still uncertain. Moreover, due to the sampling techniques used, which do not use strong sample concentration, to our knowledge no GC-mass spectrometry experiments have been reported. We report here the results obtained using GC-mass fragmentography for the analysis of F21 in atmospheric samples of country areas in the regions of Urbino and Rome.

The sampling technique, the elimination of artefacts due to impurities present in the carrier gas and trapping efficiency have been described elsewhere⁹. Between 5 and 20 l of air were passed through a stainless steel trap containing Carboxpack B, a graphitised carbon black (80–100 mesh), kept at the temperature of dry ice (about –90 °C), so that the break-through volume of F21 and other lighter halocarbons was higher than the amount of air sampled.

The GC was equipped with an oven where the trap was placed and heated to 200 °C. Injection into the column was by means of the heating-stripping technique, described elsewhere⁹.

Our major problem with the halocarbon analysis was to devise a suitable column to obtain a complete separation of all these compounds, for the reasons stated above. In particular, the separation of F21 from CH₃I is important for the determination of the F21 concentration in the atmosphere. The column used for this purpose consists of Carboxpack B 80–100 mesh coated with 0.5% SP1000, a polar acidic liquid phase. This choice was made for two reasons. First, we use the outstanding selectivity of gas-liquid-solid chromatography, where the driving force of the chromatographic process is molecular polarisability, which in several cases is independent of the vapour pressure. Second, the modifications induced by rather low amounts of a polar liquid phase do not affect the selectivity towards non-polar compounds such as halocarbons.

Furthermore, by choosing an appropriate amount of liquid phase, the separation factors may be adjusted to the particular analysis required.

Rasmussen *et al.*⁹ have reported the analysis of halocarbons trapped from 30 l of air in a suburban zone, showing that F21 is well separated from all the other halocarbons.

A major difficulty of quantitative analysis of halocarbons in the atmosphere is that some of these compounds are contained as ultra-trace impurities in high purity commercial gases. This problem has been solved by further purification of the carrier gas using a dry ice-acetone trap, and by purging the sampling traps with the purified gas before use.

Experiments have also been performed to see whether F21 could be an artefact due to sample treatment before GC analysis. A mixture of all C₁–C₂ halocarbons except F21 was inserted in the trap and stripped at 170 °C. The resulting chromatogram did not show any peak with the retention time assigned to F21.

Mass fragmentography was performed using a VG/MM 305 mass spectrometer equipped with a jet separator and a data acquisition system.

Values of *m/e* 67, 69, 83, 85, 87, were monitored and the relative abundances of these fragments were found to be consistent with the mass spectrum of F21.

An accurate determination of the atmospheric concentration of F21 is not yet possible. However, it has been estimated to range between 10 and 20 p.p.t. v/v.

Table 1 Relative concentration of F21 in the atmosphere of Rome and Urbino from GC data

Sample no.	Location	Sample vol (l)	Peak area (cm ²)	Electrometer attenuation	Relative concentration
1, 2, 3, 4	Urbino	20	1.34	×512	1.00
5, 6, 7	Urbino	10	1.62	×256	1.20
8, 9, 10	Rome	10	1.20	×256	0.97
11, 12, 13	Rome	5	0.57	×256	0.85

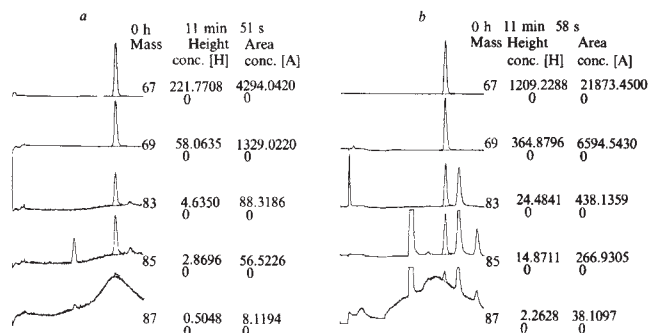


Fig. 1 Mass fragmentograms of a reference sample of F21 (a) and of the peak with the same retention time in an actual air sample (b). Computer output of the data. For details see text.

Table 1 shows the relative concentrations of F21 found in different samples from several locations. For each location the peak area is the arithmetic mean of the measurements made. Measurements within each location scatter about 20%, which is about the same as that found among measurements in different sites. Thus, it can be stated that, within the experimental errors, the concentration of F21 is fairly constant.

The computer output of the GC-MF analysis of one of the atmospheric samples is shown in Fig. 1: (a) refers to the standard F21 and (b) to the peak with the same retention time in an actual mixture of halocarbons trapped in the Urbino environment (20 l of air).

Fifteen samples trapped both in Rome and in Urbino have been analysed by GC-mass fragmentography and F21 was found to be present in each sample. The results of these experiments show that F21 is actually present in the atmosphere and cannot be considered as an experimental artefact.

This research was partially supported by EEC under contract no. 214-77-1 ENV I. We thank B. N. Green (VG Organic, Altrincham) and his staff for help with mass spectrometry.

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Received 29 January; accepted 30 March 1979.

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India and Madagascar in Gondwanaland based on matching Precambrian lineaments

THE geometrical fit of Madagascar against India has been widely accepted in most reconstructions of Gondwanaland¹. This fit is consistent with Precambrian trends, lithologies and age provinces^{2,3}. Palaeomagnetic data also support the general fit of Madagascar against India⁴, and there are suggestions that this reassembly can be recognised as far back as Proterozoic times⁵. The exact fit of east Madagascar and west India has not been adequately defined. Recent knowledge of the Precambrian geology and tectonics, has now allowed the possibility of better defining this fit, mainly on the basis of matching Precambrian re-entrant lineaments. The east coastline of Madagascar is a

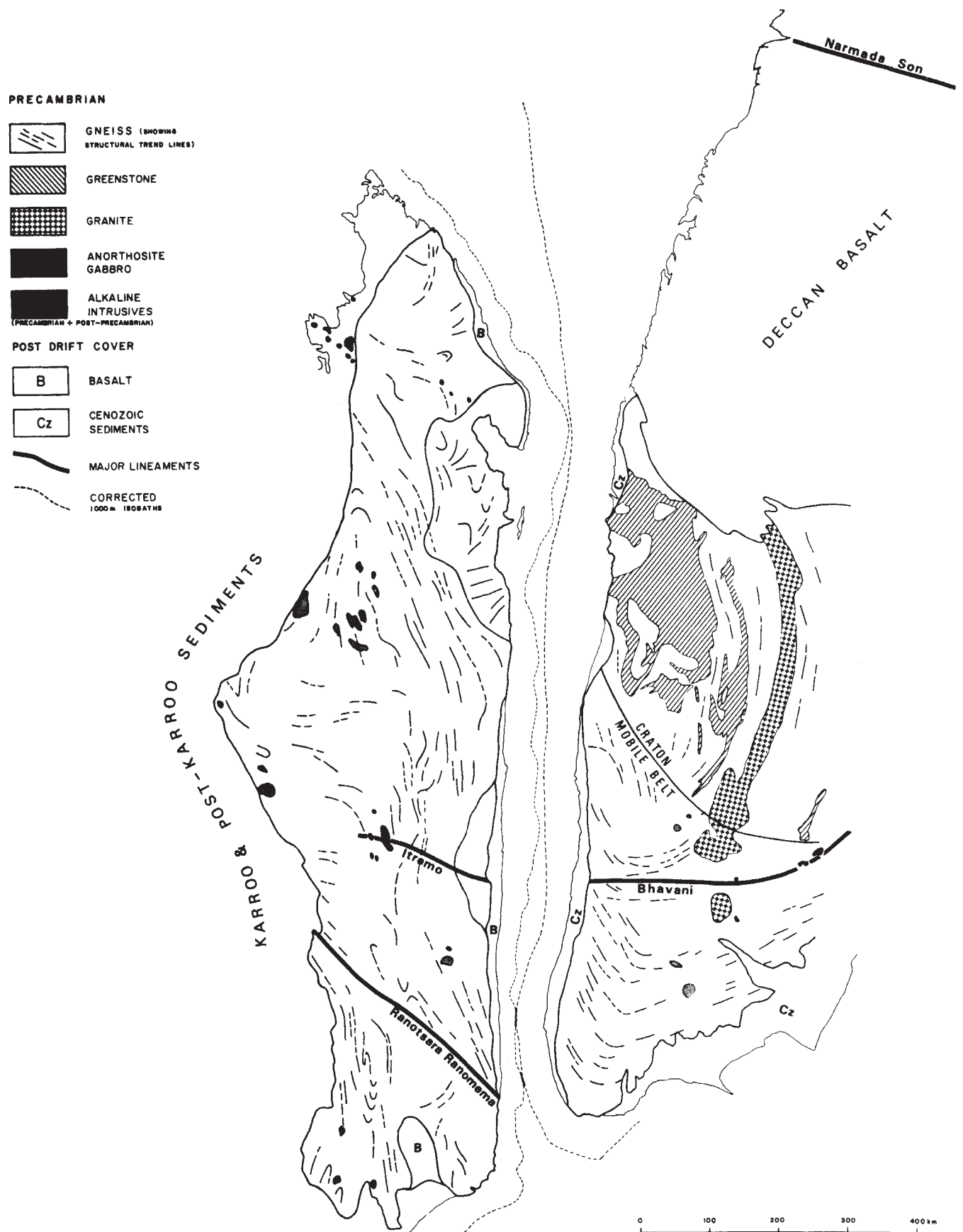


Fig. 1 India-Madagascar fit based on matching and Bhavani-Itremo lineaments. Areas in Madagascar outlined on the east coast are dated as Archaean and could possibly represent a craton.

rectilinear feature, with a narrow shelf of 25–50 km and an abrupt steep break in slope after the 1,000-m isobath. The proposed opposing coastline of west India is also rectilinear with a wider shelf of 100 km and a shelf break down to the 1,000-m isobath. The 1,000-m isobath of India was receded towards the coastline by removing the post drift sediments, according to the

best available sections⁶. A 1:2,500,000 conical projection was used to prepare a distortion-free, equidistance base map of both Madagascar and India (Fig. 1).

The Archaean terrain of south India consists of a craton, greenstone–gneiss complex surrounded by a charnockite mobile belt⁷. Basement gneisses have been dated as old as 3,200 Myr

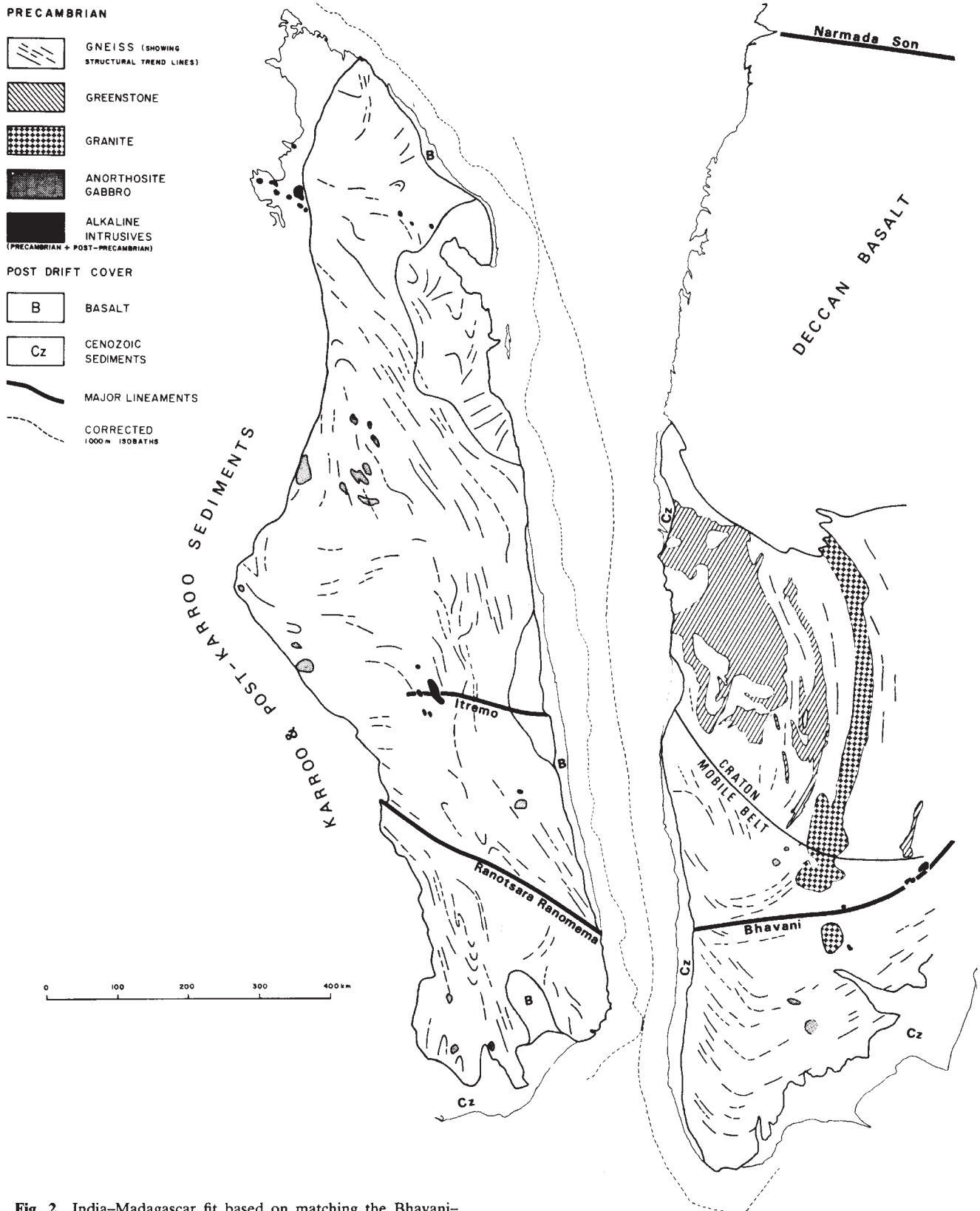


Fig. 2 India-Madagascar fit based on matching the Bhavani-Ranotsara-Ranomema lineament. The Narmada-Son lineament is continuous with a new lineament in northern Madagascar, shown here.

and old greenstones in the craton are about 3,000 Myr. Charnockite metamorphism in the mobile belt has been dated at about 2,800 Myr. Younger greenstones in the craton are about 2,600 Myr, and the granites have been dated at about 2,500 Myr. Proterozoic dates recorded in both the craton and mobile belt reflect later periodic events at about 2,000 Myr, 1,600 Myr and 900–600 Myr. The Archaean of Madagascar consists of the charnockitic complex of southern Madagascar, and a patchy distribution along the east coast^{8,9}. The widely distributed Archaean dates suggest that most Precambrian Madagascar has an Archaean history. A strong Proterozoic overprint has masked this proposed widespread Archaean event. In contrast to India, no cratonic greenstone complexes are recognised and most of the rocks appear to belong to a charnockitic mobile belt⁸. The observation that the Indian craton is girdled by a charnockitic mobile belt seems substantiated by the evidence emerging from the proposed Madagascar–Indian relationships in Precambrian Gondwanaland. The only regional lithological correlations between south India and Madagascar are a charnockitic–gneiss mobile belt containing many anorthosite–gabbro bodies, that sweep around the southern and western margins of the Indian craton. The structural trend lines of the mobile belt recognised in south India can be traced into its equivalent in Madagascar^{2,3} (Fig. 1).

From the economic viewpoint neither south India nor Madagascar are intensely mineralised. The paucity of mineral deposits recorded is not a conclusive indication of all overall poor mineral potential, as the tropical rain forest conditions and the rather attenuated exploration efforts in the past have undoubtedly contributed to a poor mining development. The metallogenesis of most of south India and Madagascar is restricted to silicates and oxides related to their common mobile belt tectonic setting. Similarly, the essentially gold–sulphide mineralisation of the Indian craton are not significantly represented in Madagascar. Several small mineral occurrences are known and occasionally exploited, and their consistence appears to be more than coincidental. Among the most obvious are the marine beach placers of monazite, zircon, ilmenite and rutile derived from the hinterland charnockitic massifs, the niobium–cerium mineralisation found associated with lineaments in Madagascar and south India, and the chromite, graphite, phlogopite and garnet occurrences.

Lineaments thought to penetrate the Indian–Madagascar parts of Gondwanaland have been used to define a fit¹⁰. The Narmada–Son lineament of northern India has been traced into northern Madagascar¹¹. Other fundamental Precambrian lineaments recently described from south India and Madagascar can be utilised to define a more exact fit. In particular, the Bhavani lineament of south India¹² is compared to two important lineaments in Madagascar, the Itremo lineament of central Madagascar^{9,13}, and the Ranotsara–Ranomena lineament of south Madagascar⁸. (Fig. 1). The fits described are compatible with the reconstruction models of Gondwanaland. The 1,000-m isobath of Madagascar is placed up against the receded 1,000-m isobath of India. This allows about 100 km of intervening continental shelf separating the opposing coastlines. Madagascar is translated relative to India along this line until the best fit is obtained in regard to geometry, bathymetry, age and lithological, structural and tectonic relationships. To define the tectonic line of reference, the Bhavani lineament was chosen. This lineament is a re-entrant feature from the Indian coastline and can be followed in a curvilinear trace to the north-east for about 500 km. The Bhavani lineament has been recognised as having an important role in the Precambrian tectonic evolution of south India¹², and also controls the emplacement of alkali intrusives, including carbonatites and ultrabasics¹⁴. A similar lineament has been described from central Madagascar, here termed the Itremo lineament^{9,13}. Several alkaline intrusions have also been recognised along the Itremo lineament. If the Itremo–Bhavani lineaments are matched, a penetrative, curvilinear feature can be traced across the Indian–Madagascar mobile belt (Fig. 1). Another possible fit is to match the Bhavani

lineament with a more distinctive lineament in south Madagascar, termed the Ranotsara–Ranomena lineament⁸ (Fig. 2). The Ranotsara–Ranomena lineament is a major discontinuity separating the southern charnockitic block from the gneiss terrain to the north. The matching of the Bhavani–Ranotsara–Ranomena lineaments also satisfies many of the geological criteria set out above.

There is not yet enough information to establish, with certainty, which fit is best. If the Bhavani–Itremo fit is selected, it defines a major lineament which apparently controlled the emplacement of alkali intrusives and carbonatites. This fit also satisfies the geometrical and bathymetrical relationships. The Ranotsara–Ranomena lineament on extension into India can control continental margin features on the south coastline of India (Fig. 1). On the other hand, the Bhavani–Ranotsara–Ranomena fit also satisfies similar criteria. The Itremo lineament could still extend into India, as similar tectonic lines have been recognised from LANDSAT as re-entrants from the coast of west India¹⁵. In addition, the Narmada–Son lineament of northern India lines up with a new lineament recognised in northern Madagascar⁹ (Fig. 2). Both of these reconstructions define major, penetrative curvilineaments that could have controlled small circle, prototransform directions in the Indian–Madagascar mobile belt, that contributed to the rifting of India from the Madagascar–African plate in Cretaceous times. The position of rifting also indicates possible control by the structural trends of the mobile belt and the presence of granulite facies charnockitic rocks¹⁶.

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Received 6 March; accepted 16 March 1979.

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Short-term climate change and New Zealand temperatures during the last millennium

MANY theories have been proposed to explain the climatic fluctuations which produced the sequence of glacial–interglacial periods of the order of tens of thousands of years which have occurred throughout the Quaternary. These fluctuations represent temperature changes in the temperate regions of about 6 °C between a glacial and an interglacial situation. There are also climatic fluctuations with a period of the order of about a thousand years representing temperature changes of about 2 °C. These changes are believed to have had an important bearing on human history, but have been little studied because it is difficult to obtain accurate temperature records for past

periods. Shorter temperature fluctuations of the order of a few decades, and representing temperature changes of perhaps as much as one degree, are of considerable economic importance and merge into the developing field of long-term meteorological forecasting. Considerable interest has recently developed in short-term climate changes. If detailed long-term climatic records could be obtained for extended periods in the past it might be possible to determine the causes for the climatic variation, and hence predict future climate trends. Even a knowledge of how hot/cold or dry/wet a particular region's climate could become, and with what probability, would be of considerable economic value to planners involved in hydro-electric development, irrigation schemes, snow clearance and the development of arid and polar regions. At present instrumental records have existed only since the mid-seventeenth century for central England, and for most other areas only over a little more than a century. Cave deposits (speleothems) provide stratigraphy which has a high inherent time resolution. Data for studying short-term temperature fluctuations can be obtained by measuring the $^{18}\text{O}/^{16}\text{O}$ ratio of suitable stalagmites¹. This technique should enable a high resolution temperature curve to be produced for many regions of the globe. We report here an investigation on the $^{18}\text{O}/^{16}\text{O}$ profile through a New Zealand stalagmite which was undertaken partly to evaluate the feasibility of obtaining high time resolution data from speleothem material and partly to compare the temperature record from New Zealand (in the Southern Hemisphere and a region meteorologically unrelated to Europe) with the English climate curve, which is the most firmly established climate curve for the last millennium. Such proxy data given us the possibility of obtaining long-term high resolution temperature records from some of the critical regions of the world where meteorological records either do not exist or are of very short duration.

Long-term climatic fluctuations such as glacial-interglacial sequences have been studied by measuring the oxygen isotopic ratios in the carbonate from the foraminifera found in deep sea cores (see, for example, ref. 2). The movements of bottom living organisms and low sedimentation rates, however, limit the time resolution obtainable from most of these cores to about 3,000 yr. It may, however, be possible to carry out similar work in suitable regions of very high sedimentation rate and to obtain data for studies of short-term temperature fluctuations at least in more recent times.

Tree rings are another possibility for obtaining high resolution palaeoclimatic information (see, for example, ref. 3). Dendrochronology provides us with stratigraphic sequences, sometimes of considerable length, which can be obtained from many parts of the world. The bristlecone pine chronology from the White Mountains in California, for example, has a length of some seven millennia⁴. The isotopic-geochemical problems are formidable but a time resolution better than one year and absolute dating system accurate to one year could be obtained.

The work described here exploits the stratigraphy present in cave deposits where the chemistry is simple but the dating is less certain than with tree rings. It may eventually be possible to combine information from both sources and use the tree-ring record to confirm and correct the speleothem time scale.

If it can be assumed that a stalagmite has been laid down in isotopic equilibrium with the water then the oxygen isotope ratio of its calcite is controlled by two factors¹: (1) the isotopic composition of the water flowing into the cave; and (2) the temperature at which the calcite is deposited on the speleothem, which for many caves can be taken as the mean annual temperature. The isotopic fractionation of the oxygen isotopes between calcite and water will increase by 0.024‰ as the temperature of deposition decreases by 1 °C. As existing mass spectrometric techniques enable relative measurements of $^{18}\text{O}/^{16}\text{O}$ to be made to 0.0025%, one should be able to obtain palaeotemperature curves to 0.1 °C and this should be useful for studying short-term climatic fluctuations.

The $^{18}\text{O}/^{16}\text{O}$ composition of the atmospheric precipitation percolating into the cave is determined first by the isotopic

composition of the oceans. This changes between the glacial and interglacial situation due to the removal of water depleted in ^{18}O and its deposition on the ice sheets; however, this effect is negligible for the short-term temperature fluctuations in the present study. The second effect is caused by the temperature difference between the region of evaporation and the area under study. This effect tends to work in the opposite direction to the effect of temperature and hence would tend to reduce any fluctuations due to temperature. However, if tropical stalagmites are measured they should give temperature changes of the tropics directly. Further comparison between stalagmites from tropical and temperate regions will give a plot of changes in temperature differences during time and hence a record of past latitudinal temperature gradients. Such data could provide a measure of the intensity of the zonal atmospheric circulation in the past and throw light on such problems as loess deposition and sand dune formation and even provide interesting archaeological information.

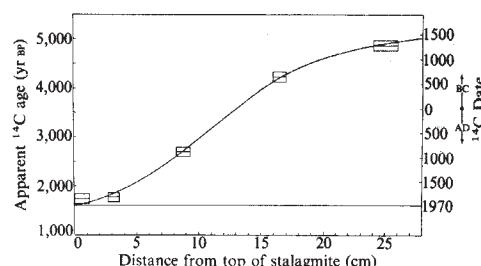
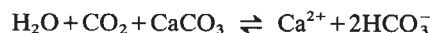


Fig. 1 ^{14}C data from which the stalagmite was dated. Horizontal bars represent areas of the stalagmite sampled. Vertical bars represent $\pm$ one standard deviation of the ^{14}C determination.

Gathering any palaeoclimatic data from stratigraphy involves some dating method. In the case of speleothems this can be achieved for the past 35,000 years by ^{14}C dating. However, the carbon laid down as carbonate on a stalagmite contains a mixture of ancient carbon from the limestone carbonate, which contains essentially no ^{14}C , and modern carbon respired by plant roots. The calcium in solution has been dissolved from the limestone according to



Assuming that saturation is reached as the CO_2 solution percolates through the limestone and into the cave then a little more than half the carbon in the resultant solution would be of recent biogenic origin and a little less than half derived from limestone⁵. This theoretical situation is not always the case (for example, the Twin Forks stalagmite). This is due to the exchange of carbon dioxide with cave air. Hendy⁵ has described how such corrections can be made using $^{13}\text{C}/^{12}\text{C}$ ratios. In the case of a stalagmite which is still growing when collected, an even more accurate estimate can be made by determining the ^{14}C content of the outer layer of the stalagmite. Other methods such as thermoluminescence are also potentially useful for dating a material such as calcite. Several samples must be dated across the stalagmite to correct for any variation in growth rate of the stalagmite during the time under investigation.

We examined a stalagmite from a cave in north-west Nelson lat 40°40' S, long 172°26' E and growing at an altitude of about 50 m above sea level. The stalagmite was taken near a meteorological station which has a present-day mean temperature of 12.2 °C (ref. 6). The stalagmite was sectioned and 30-g samples taken for ^{14}C determination to provide a time base. These data are presented in Fig. 1. Samples (~50 mg) were also taken at regular intervals down the axis of the stalagmite for isotopic analysis with a 1.6-mm steel drill. Five 10-mg aliquots of each of the samples of calcite were reacted with 100% phosphoric acid at 25.0 ± 0.1 °C in evacuated glass reaction vessels. The carbon dioxide was purified⁷ and the ratios of mass

45 to (mass 44 + mass 46) and mass 46 to (mass 44 + mass 45) were compared with a sample of carbon dioxide prepared from Te Kuiti limestone⁸ on a Nuclides Analysis Associates 60° double collector mass spectrometer. The $^{18}\text{O}/^{16}\text{O}$ ratio is reported as $\delta^{18}\text{O}$ with respect to the international standard PDB⁸, where

$$\delta^{18}\text{O}(\text{‰}) = \frac{^{18}\text{O}/^{16}\text{O}_{\text{(sample)}} - ^{18}\text{O}/^{16}\text{O}_{\text{(PDB)}}}{^{18}\text{O}/^{16}\text{O}_{\text{(PDB)}}} \times 1,000$$

The mean values of the $\delta^{18}\text{O}$ are plotted in Fig. 2, the error bars represent one standard deviation of each particular set of measurements. In such a study the $^{13}\text{C}/^{12}\text{C}$ ratio must be plotted against the $^{18}\text{O}/^{16}\text{O}$ ratio to ensure that they do not correlate and hence confirm that the stalagmite was laid down in conditions of isotopic equilibrium⁵. The results of these data are combined as a palaeotemperature curve on Fig. 3 and compared with Lamb's curve for central England⁹.

The curve has been scaled in terms of temperature as we know the temperature at which the stalagmite was growing when collected and the temperature change for the last cold period in New Zealand. This cold period occurred during the first decade of this century and was recorded by direct instrumental observations. As the above estimate agrees well with the known temperature dependence of the fractionation factor of calcite, it suggests that little or no change in the groundwater composition has taken place and the isotope fluctuations in the stalagmite studied seem to be mainly due to temperature changes.

These very preliminary results mainly investigate the potential of stalagmites to study short-term temperature variations. Clearly, many stalagmites should be taken from different caves in different parts of New Zealand. However, the temperature curve for New Zealand is apparently broadly similar to England and such climatic fluctuations as the Mediaeval Warm Period and Little Ice Age are not just a local European phenomenon.

It seems from the temperature curve that the cooling in New Zealand has been delayed and is generally more rapid than in central England. This may be caused by dating errors. The temperature is known better than the actual date of the New Zealand curve whereas the date is known accurately from historical records but the temperature may be in error for the English curve. However, the past 100 yr of the curve agree well with New Zealand meteorological records¹⁰ and not the English temperature curve during the same period. Whereas the temperature of England since the 1940s has fallen significantly¹³ the mean New Zealand temperature is still climbing¹⁰.

An interesting feature of the curve is that in recent times samples representing times as short as 10 yr were measured. Because the hole drilled in the stalagmite is circular in section more calcite is obtained from the central years of the time span

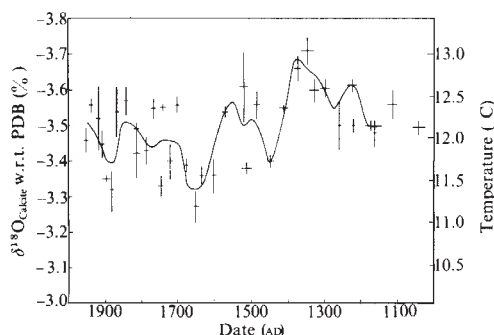


Fig. 2 $^{18}\text{O}/^{16}\text{O}$ profile through the stalagmite expressed in % deviation from the international isotope standard PDB (Pee Dee Belemnite). The temperature scale on the left has been estimated using the modern day mean temperature for the region from which the stalagmite came and the known temperature fractionation of the calcite water system. The error bars represent one standard deviation of each particular set of measurements. The solid curve is the 50-yr running mean.

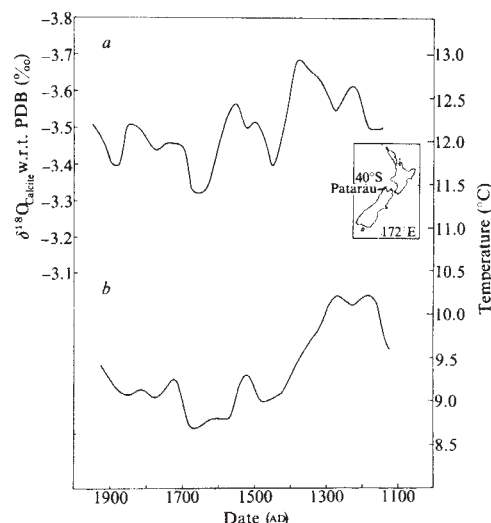


Fig. 3 The 50-yr running mean curve (a) from Fig. 2 is compared with that from central England (b) given by Lamb, ref. 9.

with progressively less as the extremes are approached. A limiting factor to the time resolution of the technique is that winter precipitation is more depleted in ^{18}O than summer precipitation. If there is more winter growth than summer or vice versa the point will be displaced upwards or downwards respectively. This effect only becomes significant when a small number of years is sampled. The problem could be overcome by drilling out many samples along a growth line and mixing them. However, this study shows that the technique is capable of a time resolution of only a few years and is apparently limited only by the winter/summer sampling problem. As can be seen from the New Zealand temperature curve, the very rapid temperature drop in the fourteenth century may well have had a catastrophic effect on the Polynesian agriculture, based as it was on the tropical food plants (taro and kumera) in all except the very north of the North Island. It is probably no coincidence that the Polynesian exploration of the South Pacific came to an end at this time. As has been discussed in an earlier paper¹⁴ a cooling might be expected to lead to steeper latitudinal temperature gradients and hence to a more violent climate. The loss of travellers by the increase in frequency of more violent storms would effectively discourage further exploration.

We thank the New Zealand Institute of Nuclear Science for use of their mass spectrometer and for financial support to C.H.H.

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Received 25 November 1978; accepted 3 April 1979.

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Effect of rapid precipitation of dissolved Mn in river water on estuarine Mn distributions

ALTHOUGH the general characteristics of estuarine chemical behaviour can be deduced from distributional data¹, there is an urgent need for greater understanding of the mechanisms and kinetics of the processes involved to assist the development of precise geochemical cycling models and to allow accurate predictions of the consequences of waste disposal into estuaries and inland waters. Manganese is of particular importance in this respect because, as well as exhibiting pronounced reactivity within estuaries²⁻⁴, the highly absorptive properties of particulate and colloidal manganese oxide phases may contribute significantly to the estuarine behaviour of many other trace metals⁵. We show here how measurements of the effects of suspended particles and salinity on the rate of precipitation of dissolved manganese can lead to an explanation of the temporal and geographical variability in the distribution of dissolved manganese in an estuarine system.

During investigations of the distribution of manganese in the Tamar Estuary, South-west England, we have occasionally encountered a minimum in dissolved manganese (which can fall below $1 \mu\text{g l}^{-1}$) in the freshwater immediately above the salt wedge. Such minima invariably coincide with high concentrations of suspended particulates produced by intrusion of the estuarine turbidity maximum into the freshwater. Upstream of the limit of this intrusion, dissolved manganese concentrations are usually within the range $20\text{--}60 \mu\text{g l}^{-1}$. These results indicate a rapid removal of dissolved manganese by the suspended particles. A study of this process has demonstrated zero-order reaction kinetics with respect to dissolved manganese in freshwater and has provided evidence of the mechanisms involved.

All experiments were carried out on samples of natural water obtained from the river and incubated in the dark at constant temperature. Dissolved manganese concentrations (passing a $0.45 \mu\text{m}$ pore-sized membrane filter) were determined using the flameless atomic absorption procedure of McArthur⁶ and the weight of suspended particulate material (dried at 110°C) was measured gravimetrically. Experimental manipulation of suspended particulate concentration was achieved by mixing proportions of natural river water, collected either from within

or from upstream of the zone of maximum turbidity, with water which has been filtered through a $0.45 \mu\text{m}$ membrane filter.

A rapid loss of dissolved manganese in natural river waters was obtained during these experiments which was kinetically of zero-order with respect to the concentration of dissolved manganese (see Fig. 1). In contrast, removal of dissolved manganese from estuarine waters approximated to first-order with respect to dissolved manganese and was considerably slower. The rate of removal decreased with increasing salinity. The zero-order rate law applied to samples of river water whether or not the particulate material originated from within the estuarine turbidity maximum, for particulate concentrations in the range $0.5\text{--}500 \text{ p.p.m.}$, and at temperatures within the range $5\text{--}20^\circ\text{C}$. Increasing temperature always accelerated the reaction. No detectable loss of manganese was apparent in samples from which the particulate materials were removed by filtration and the rate of removal increased with increasing particulate load. However, it was generally evident that lower particulate concentrations exerted a disproportionately large effect, particularly at higher temperatures. Furthermore, the rate of manganese removal varied significantly between experiments carried out at different times but with equivalent concentrations of particulate material. Using the Arrhenius equation to calculate the apparent activation energy of the removal process has consistently yielded low values, usually within the range $5\text{--}15 \text{ kcal mol}^{-1}$. The combined effects of temperature and the concentration of particulate material on the zero-order removal rate for a typical experiment are shown in Fig. 2.

Morgan's⁷ extensive investigations would indicate that, for an oxidative reaction within an initially homogeneous solution corresponding in composition to the river waters under investigation, the reaction should, at first, proceed with first-order kinetics with respect to divalent manganese, but would be extremely slow. The oxidative reaction is, however, subject to pronounced autocatalysis generated by manganese oxide reaction products^{7,8}. Other naturally-occurring particulate or colloidal surfaces, for example, silica⁹, feldspar¹⁰, hydrated aluminium and iron oxides⁵, seem to catalyse the oxidation although their catalysis may well be due to, or enhanced by, the presence of manganese oxide surface 'impurities'. Furthermore, manganese oxides, particularly if freshly precipitated, are highly efficient and to some extent specifically adsorptive and/or coprecipitative scavengers of certain metal ions, including divalent manganese^{11,12}. Taking these properties into account, Crerar and Barnes¹³ and Hem^{14,15} have proposed that coupled specific adsorption-catalysed oxidation reactions occurring at the surface of pre-existing manganese oxide solids, with consequent regeneration of the reaction sites, can explain the continuous accretion of hydrogenous manganese oxide solid phases.

The kinetic results reported here are in accordance with such a mechanism. By analysing both dissolved and particulate manganese during a kinetic experiment, we have found that the reaction products are quantitatively retained by the particulate phase, although the kinetic results show that the catalytically effective property of the particles is conserved during the reaction. This agrees with the self-regenerating properties inherent in the mechanism noted above, and indicates that pre-existing manganese or mixed manganese-iron oxide phases occurring as superficial coatings on the particles act as self-maintaining catalytic sites for continued uptake of dissolved manganese. The indifference of reaction rate to the dissolved manganese concentration, that is zero-order kinetics, implies sorptive occupation of all available reaction sites by the reactant throughout the course of the reaction. This is possible if the rate of attainment of sorptive equilibrium for divalent manganese on the catalytic phase is considerably faster than the overall reaction rate. Experimental sorption studies¹¹ indicate that this is so. The slower, first-order reaction characteristic of estuarine waters can be attributed to a significant competition for occupation of the catalytic sites exerted by the major seawater cations.

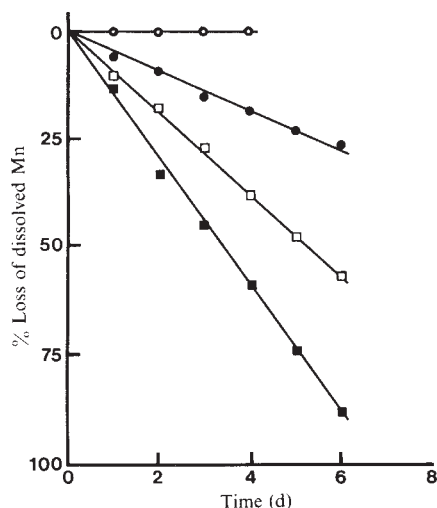


Fig. 1 Loss of manganese from solution in Tamar River water containing 0% (○); 10% (●); 50% (□); and 100% (■) of the original particulate load during incubation at 5°C . The water originally contained $47 \mu\text{g l}^{-1}$ of dissolved manganese and 60 p.p.m. (w/v) suspended solids.

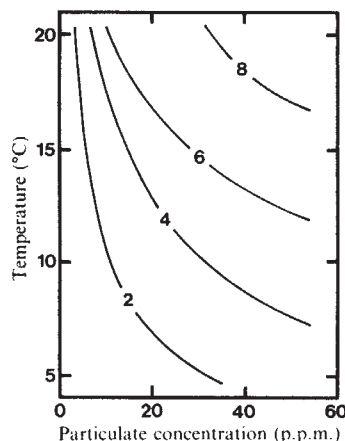


Fig. 2 Rate of zero-order loss ($\mu\text{g l}^{-1} \text{d}^{-1}$) of dissolved manganese from solution in Tamar River water as a function of temperature and particulate load.

Temporal variations in the rate of manganese removal per mass of suspended particles are probably caused by an inherent natural variability in the surface characteristics of the particulates. The non-linearity of the relationship with respect to particulate concentrations at any one time is probably induced by increased aggregation of particles at higher concentrations of particulate material.

Although occurring within freshwater medium, the very rapid transfer of dissolved manganese to the particulate phase is an estuarine-induced phenomenon, in that the necessarily high concentrations of particles in the water column are generated and maintained by estuarine physical processes¹⁶. In the Tamar Estuary, cyclic sping-tide to neap-tide variability in the average amount of particulates comprising the turbidity maximum has been found to impart a corresponding variability in the rate and extent of removal of dissolved manganese in the freshwater immediately above the salt wedge. Furthermore, during neap-tide to spring-tide periods of increasing tidal amplitude, with pronounced net mobilisation of sediment, the removal of dissolved manganese may be more than counterbalanced by the infusion of sediment pore water containing relatively high concentrations of divalent manganese. Consequently the concentration of dissolved manganese in the freshwater entering the estuarine mixing sequence undergoes cyclic oscillation. This provides an alternative and/or complementary explanation for the common occurrence of a dissolved manganese maximum within estuarine water^{2-4,17,18}. These maxima are usually attributed to desorption of manganese from riverine particles or to advective and diffusive influxes from the estuarine sediment although such explanations do not always seem to be quantitatively adequate².

This work forms part of the Estuarine Ecology Programme of the Institute for Marine Environmental Research, a component of the Natural Environment Research Council, and was partly supported by the Department of the Environment on contract no. DGR 480/48.

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Received 2 March; accepted 9 April 1979.

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Amino acids in interstitial waters of marine sediments

DISEQUILIBRIA within the mixture of organic matter, mineral particles and seawater in near-surface sediments result in extensive reaction, often biologically mediated. Variations in interstitial water composition are very sensitive indicators of the chemical and biological reactions in this zone of transition and the interaction between the water column and the permanent deposit^{1,2}. Although most studies have been confined to inorganic ions, the few measurements of pore water DOC in marine sediments³⁻⁶ show large gradients in dissolved organic matter concentration within sediments and across the sediment-water interface. We describe here our study of dissolved amino acids in interstitial waters, which were used to trace some of the biogeochemical processes affecting organic matter in marine sediments. We have analysed the dissolved free amino acids in 15 interstitial water samples from four cores obtained in Buzzards Bay, Massachusetts; the Gulf of Maine; and the North-west Atlantic continental rise. These pore waters have very high amino acid concentrations, of the order of 1 mg l^{-1} . In addition, the distribution of individual amino acids differs substantially from that reported for seawater^{7,8}, particularly in the large relative abundance of glutamic acid and β -aminoglutaric acid. β -aminoglutaric acid ($\text{HOOCCH}_2\text{CH}(\text{NH}_2)\text{CH}_2\text{COOH}$) is an isomer of glutamic acid which, to our knowledge, has not been previously reported in the marine environment.

Sampling was carried out using a 20 cm diameter sphincter corer⁹ in Buzzards Bay and a 0.25 m^2 box corer (Sandia-Hessler Type MK III) in the Gulf of Maine and the North-west Atlantic. The Buzzards Bay (station P) sediment sampled was located in 17 m of water and was anoxic below the bioturbated upper 1-3 cm. Station 3 and station 8 in the Gulf of Maine were located at 250 m depth in the Wilkinson Basin and at 390 m in the Georges Basin, respectively. A small amount of H_2S was present in the deepest (24-28 cm) section at station 3, but none was detected in the 10 cm core from station 8. Station 10 was located on the continental rise east of the Gulf of Maine at a depth of 4,200 m. The sediment sampled was a strongly oxidising, foram-rich ooze. Cores were sectioned immediately and the sediment subsamples refrigerated until squeezing was completed (within 24 h). Pore water was extracted from 100 to 200 g sediment using a hydraulically-powered stainless steel squeezer with two internal precombusted Reeve Angel^(R) glass fibre filters and then refiltered through Gelman Type A^(R) glass fibre filters (nominal particle diameter retained $0.3-1 \mu\text{m}$). The filtered water and samples of whole sediment from corresponding core sections were frozen for onshore analysis.

After returning to Woods Hole, 10-20 ml pore water aliquots were applied to a 15 cm^3 Bio-Rad^(R) AG 50W-X8 resin cation exchange column (H^+ form) for desalting. The column was eluted with 25 ml water and then $1.5 \text{ M NH}_4\text{OH}$ until the eluate began to become basic. The first 60 ml of the basic eluate containing the amino acids were collected, evaporated to dry-

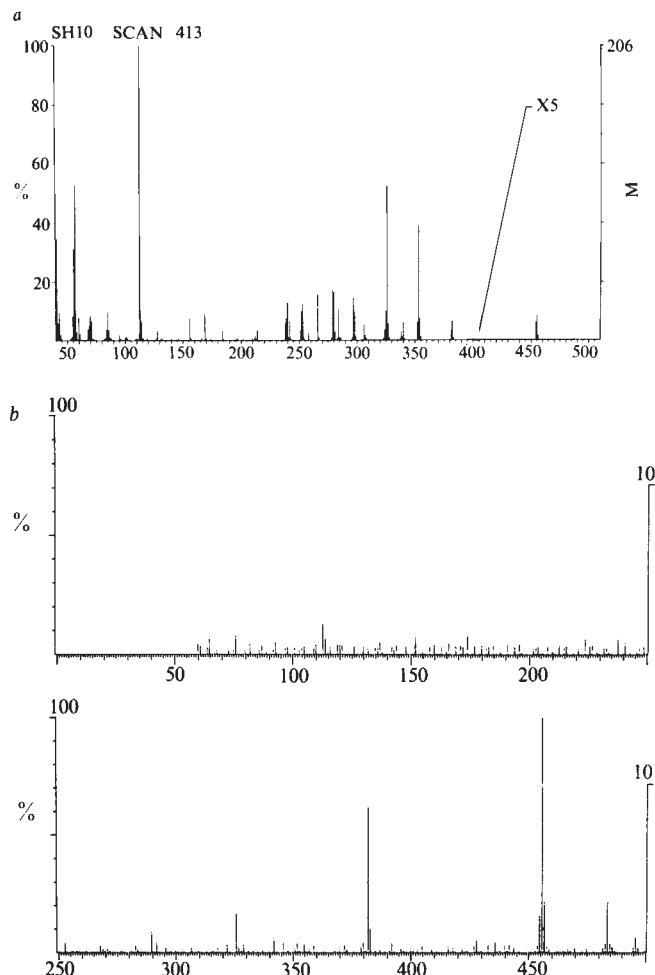


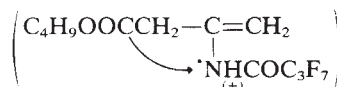
Fig. 1 *a*, Electron-impact ionisation mass spectrum of β -aminoglutaric acid from station 3, 0–2 cm core section interstitial water; SE-52 glass capillary GC column interfaced with a Finnigan GC-MS 3200, ionisation voltage 70 eV. *b*, Chemical-ionisation mass spectrum of β -aminoglutaric acid; SE-52 glass capillary GC column interfaced with a Finnigan GC-MS 1015 SL, CH_4 reactant gas, ionisation voltage 130 eV.

ness, and the residue derivatised to form the *N*-heptafluorobutyl *n*-butyl esters¹⁰ for gas chromatographic (GC) analysis. Blanks of the entire analytical procedure, including rinses of the squeezer and filters, give negligible concentrations of amino acids.

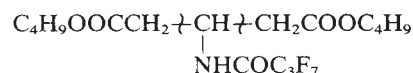
Gas chromatographic analyses were carried out on a 32 m $\times$ 0.3 mm i.d. SE-54 glass capillary column. The column was installed in a HP 5840 gas chromatograph with splitless injection at a column temperature of 40 °C and an injector temperature of 225 °C. After four minutes, the temperature was increased at 30 °C min⁻¹ to 70 °C, and then at 3 °C min⁻¹ to 240 °C. The carrier gas flow rate was 2–3 ml He min⁻¹ and the FID temperature was 250 °C. Derivatives of cysteine, histidine, and tryptophan were not detected in these conditions, and asparagine and glutamine were measured as aspartic and glutamic acids, respectively. Selected samples were subjected to gas chromatography-mass spectrometry (GC-MS) (Finnigan GC-MS 3200 or Finnigan GC-MS 1015 SL). Compounds were identified by comparison to GC retention times and mass spectra of amino acid standards (Sigma) obtained in the same conditions and with the same instruments used for samples.

β -Aminoglutaric acid (which may occur to some extent as the glutamine-isomer amide) is a major amino acid in our pore water samples, making up 2–50% of the dissolved free amino acids. The chemical ionisation (CI- CH_4) and electron-impact ionisation (EI) mass spectra of the interstitial water amino acid (as the *N*-heptafluorobutyl *n*-butyl ester) which we have identified as β -aminoglutaric acid are

given in Fig. 1. The molecular ion at m/e 455 (confirmed by the presence of $M+1=456$, $M+29=484$, and $M+41=496$ in the CI- CH_4 spectrum) indicates that the compound is an isomer of glutamic acid. The greater intensity of m/e 353 ($=M-102$) relative to m/e 354 is characteristic of a β -amino acid rather than an α -amino acid¹¹. The m/e 326 ion (probable structure $\text{C}_4\text{H}_9\text{OOCCH}=\text{NHCOC}_3\text{F}_7$) may arise subsequent to a rearrangement of the m/e 353 ion



The base peak at m/e 113 probably results from cleavage β to the amide N with loss of hydrogen:



The EI and CI- CH_4 mass spectra and glass capillary GC retention time of authentic β -aminoglutaric¹² acid are the same as those of the unknown compound in our samples. Those of the next most likely isomers of glutamic acid, α -methylaspartic and β -methylaspartic acids, are markedly different.

Dissolved free amino acid concentrations in interstitial water are given in Table 1. Total concentrations in the samples analysed, ranging from 0.82 to 5.6 mg l⁻¹, are two orders of magnitude greater than those found in nearshore waters, 5–30 $\mu\text{g l}^{-1}$ (ref. 7) or in open ocean seawater, 0–4 $\mu\text{g l}^{-1}$ (ref. 8). Concentrations generally reach a maximum within the upper 6 cm and then decrease with depth. Glutamic and β -aminoglutaric acids make up more than 90% of the dissolved free amino acids in station P interstitial waters, and from 76 to 90%

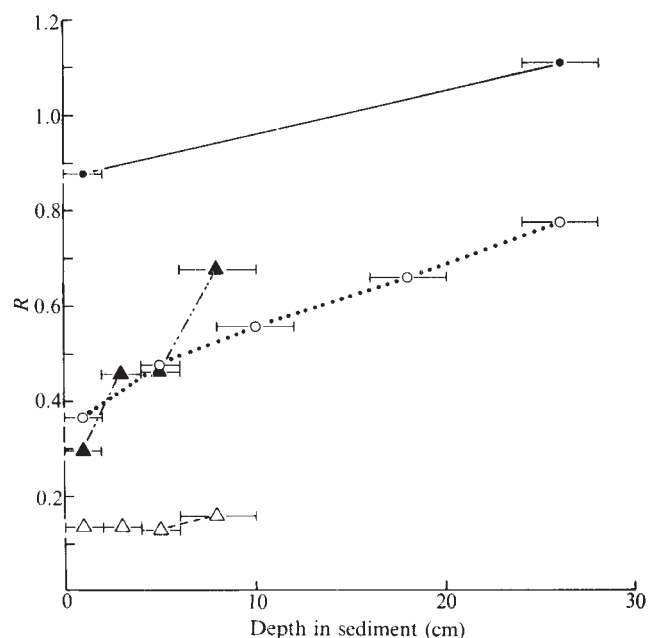


Fig. 2 *R* (β -aminoglutaric acid/glutamic acid ratio): variation with depth in sediment and with overlying water column depth. ●, Station P (17 m); ○, station 3 (250 m); ▲, station 8 (390 m); △, station 10 (4,205 m).

of the total at station 3. Station 8 pore waters have significantly greater concentrations of other amino acids, especially alanine, glycine and serine, relative to glutamic and β -aminoglutaric acids which make up 40–75% of the total. Station 10 has a more uniform distribution of the protein amino acids and lower relative abundances of glutamic and β -aminoglutaric acids (18–35%).

Surface sediments from stations 3, 8, and 10 were hydrolysed in 6M HCl and the amino acids were analysed as described above. Concentrations range from 1.3 to 5.6 mg per g dry weight (Table 1), decreasing with overlying water column depth. The

Table 1 Amino acid concentrations in interstitial waters* and sediments†‡

	Ala	Gly	Val§	Thr	Ser	Leu	Ile	Pro	Asp	Phe	Glu	β Glu	Lys	Tyr	Total
Interstitial water															
Station P															
0–2 cm	36	19	— [¶]	—	23	13	—	—	37	—	820	720	—	—	1.7
24–28 cm	24	57	—	—	—	—	—	—	70	—	620	680	—	—	1.4
Station 3															
0–2 cm	66	140	28	18	48	17	13	23	110	15	1,100	400	—	14	2.0
4–6 cm	59	42	39	28	40	33	19	10	81	20	1,800	860	—	—	3.1
8–12 cm	52	71	102	81	67	—	—	—	83	—	1,200	670	—	—	2.4
16–20 cm	28	28	14	32	49	—	—	—	40	—	630	410	—	—	1.2
24–28 cm	16	13	—	—	23	—	—	—	29	—	420	320	—	—	0.82
Station 8															
0–2 cm	360	1,500	360	85	110	120	82	110	260	75	1,600	480	—	81	5.2
2–4 cm	190	340	46	96	340	38	34	34	150	23	1,700	760	—	40	3.8
4–6 cm	400	990	73	44	110	81	57	30	94	33	1,300	560	—	34	3.8
6–10 cm	58	110	27	25	93	21	—	—	44	—	660	440	—	—	1.5
Station 10															
0–2 cm	490	480	320	190	210	240	220	50	420	60	940	130	—	18	3.7
2–4 cm	900	170	170	200	110	210	160	16	190	66	610	90	—	19	2.9
4–6 cm	1,500	780	360	290	210	480	360	57	330	170	880	110	—	97	5.6
6–10 cm	130	310	67	35	49	64	55	17	74	31	390	60	—	16	1.3
Sediment hydrolysate															
Station 3															
0–2 cm	0.63	0.83	0.49	0.42	0.43	0.32	0.20	0.26	0.89	0.22	0.54	—	0.23	0.06	5.6
Station 8															
0–2 cm	0.19	0.20	0.15	0.15	0.16	0.12	0.073	0.069	0.24	0.069	0.12	—	0.039	0.017	1.6
Station 10															
0–2 cm	0.14	0.18	0.077	0.10	0.11	0.078	0.043	0.056	0.16	0.047	0.098	—	—	—	1.3

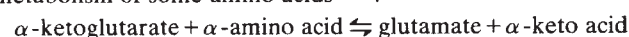
* Interstitial water dissolved free amino acid concentrations in $\mu\text{g l}^{-1}$, with depth-interval totals in mg l^{-1} .† Sediment hydrolysate concentrations in $\text{mg per g dry weight}$.‡ Amino acid abbreviations are standard, except $\beta\text{Glu} = \beta\text{-aminoglutaric acid}$.§ Peak sometimes had shoulder or partially resolved component which may be $\beta\text{-alanine}$.

|| Peak sometimes had shoulder or partially resolved component, probably due to small amounts of lysine.

¶ $<10 \mu\text{g l}^{-1}$ for interstitial water and $<0.01 \text{ mg per g dry weight}$ for sediment hydrolysate. Some minor components, including hydroxyproline, $\gamma\text{-aminobutyric acid}$, ornithine and $\alpha\text{-aminoadipic acid}$, were omitted from this table.

amino acid compositions of the sediment hydrolysates are similar to those found by others for organic-rich near-shore sediments^{13–15}. $\beta\text{-Aminoglutaric acid}$ is present only in trace amounts ($<0.4\%$ of the total hydrolysable amino acids), and glutamic acid makes up $<10\%$ of the total. Thus, if pore water amino acids are supplied from detrital proteins in sediments, for example by bacterial proteolysis, either the process is selective for particular residues or proteins of very unusual composition, or the freed amino acids are further metabolised to produce the observed distribution.

The large relative abundance of glutamic acid in interstitial water may result from the following transamination which many organisms, including bacteria, carry out as a first step in the metabolism of some amino acids^{16,17}:



Another potential source for dissolved free amino acids in interstitial water is excretion or loss of cellular fluids by higher benthic organisms. The magnitude and even the direction of the net flux of amino acids to and from benthic organisms such as polychaetes is the subject of some controversy^{18,19}, and there is little information on the composition of these fluxes. Thus the significance of this source of amino acids cannot yet be evaluated.

The large relative abundance of $\beta\text{-aminoglutaric acid}$ in pore waters is surprising, because we have been unable to find any reference to it as a natural product in the literature. In the 15 samples we have analysed so far, the $\beta\text{-aminoglutaric acid}$ concentration is correlated ($r = 0.81$) with the concentration of glutamic acid. In contrast, glycine, another abundant amino acid in some of our samples, shows no significant correlation. However, the levels of $\beta\text{-aminoglutaric acid}$ do not depend solely on those of glutamic acid, as can be seen from Fig. 2, which plots the $\beta\text{-aminoglutaric/glutamic acid ratio (R)}$ in pore water against depth in core. R increases with depth in all four cores. Furthermore, R is non-zero in surface sediments, and this surface ratio decreases with increasing water depth from station P through stations 3 and 8 to station 10.

The concentration correlation between glutamic acid and $\beta\text{-aminoglutaric acid}$ in pore water and the increase of R with depth in sediment suggests that $\beta\text{-aminoglutaric acid}$ is being formed from glutamic acid. The fact that substantial amounts of $\beta\text{-aminoglutaric acid}$ are present in surface sediments indicates that the reaction proceeds very rapidly and is probably biologically mediated. However, we have found no indication that such an $\alpha \rightarrow \beta$ isomerisation is carried out by bacteria or other organisms except during lysine fermentation by certain *Clostridia*¹⁷. On the other hand, the inputs of $\beta\text{-aminoglutaric acid}$ and glutamic acid may be by separate, but related processes. The increase in R with depth in core might then be explained by preferential metabolism or reduced inputs of glutamic acid rather than its conversion to $\beta\text{-aminoglutaric acid}$. In either case the decrease in the surface R from the shallow to the deep-water sediments is probably related to some characteristic(s) of the sedimentary environments, for example, redox potential and the related nature of the benthic community metabolising organic matter. We are currently analysing additional samples from a wider range of sediment types to improve our understanding of the relationship between pore water amino acid concentration and composition and sedimentary environment.

This research was supported by Contract N00014-74-C0262 NR 083-004 from the Office of Naval Research and the Paul M. Fye Fellowship awarded through the WHOI Education Program to S.M.H. We thank Drs Nelson Frew and Philip Gschwend for obtaining mass spectra and Drs Cindy Lee, Robert Gagosian, Oliver Zafiriou, Fred Grassle and Werner Deuser for reviewing the manuscript. Authentic $\beta\text{-aminoglutaric acid}$ was provided by Drs Alton Meister and Daniel Purich.

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Reduced thermogenesis in obesity

It is often claimed that there are obese patients who find it difficult to maintain a normal body weight because they have such low energy requirements that even normal intakes of energy result in weight gain and obesity. Studies of both children¹ and adults² show that there can be a twofold difference in energy intake between individuals despite apparently similar patterns of physical activity. An individual variability in the capacity to dissipate heat by metabolic changes has therefore been suggested³ but no physiological basis for the differences in thermogenesis has yet been established. In genetically obese *ob/ob* mouse there are two components involved in the deposition of excess body fat: hyperphagia and increased metabolic efficiency^{4,5}. Metabolic efficiency is the major factor responsible for obesity when the animals are kept at 20 °C so these animals provide a model study of the link between metabolic rate and obesity. Pre-obese and obese *ob/ob* animals have an abnormality of thermoregulatory thermogenesis with a reduced thermogenic response to cooling⁶. A defect in non-shivering thermogenesis can be confirmed by monitoring the thermogenic response to maximum doses of noradrenaline: the *ob/ob* mouse has only half the response of its lean littermate. The abnormal thermoregulatory thermogenesis quantitatively accounts for most of the metabolic efficiency of the obese animals as pair feeding at thermoneutrality rather than at 23 °C reduces the excess fat deposited by 65%⁷. We report here that obese adults with a family history of obesity have a reduced metabolic response to noradrenaline infusion compared with thin adults. As the reduced non-shivering thermogenesis is also found in subjects with familial obesity who remain at normal weight by persistent dieting, the defect in non-shivering thermogenesis appears to be constitutional and not a secondary consequence of obesity.

Thermogenic responses were tested in six obese women, aged 47 ± 4.5 yr (mean $\pm$ s.e.m.), who claimed to gain weight readily and who had a strong family history of obesity. A group of seven lean women of equivalent age (49.5 ± 2 yr), with no family history of obesity, served as controls. All seven claimed that they were able to eat freely and had no problems with weight gain. A third group of 'post-obese' women (aged 44.2 ± 5.4 yr) was also studied once they had slimmed from an obese state after 1–2 yr on a severely restricted diet. They had maintained a stable weight by careful dieting for at least 3 months before the test. The subjects in all three groups were euthyroid and normotensive.

The metabolic capacity for thermogenesis was tested by infusing noradrenaline intravenously in a dose related to the ideal body weight (IBW). Tests were conducted at the same time

of year with all subjects in a weight-stable condition. Subjects were fasted overnight for 12 h and were tested in identical clothing in thermoneutral conditions (27.2–27.6 °C) after allowing 1 h for heat equilibration.

The resting metabolic rate (RMR) was measured at 1-min intervals for 30 min after the subject had rested supine for 30 min. Noradrenaline (Levophed, Winthrop) was then infused intravenously (0.1 μ g per kg IBW per min) through a brachial vein for 45 min. The intravenous dose of noradrenaline was chosen to produce in the other arm a venous plasma noradrenaline corresponding to that found in moderately severe exercise. An indwelling Abbott 19-gauge venous cannula, kept patent with 3.8% sodium citrate (BPC) and inserted in the non-infused arm, was used to remove blood samples for assay. The RMR was measured continuously during the infusion and for 45 min after the infusion had stopped.

Immediately on starting the infusion of noradrenaline, the RMR increased and remained at near plateau levels for the duration of the infusion (Fig. 1). The rise in the RMR in the lean group was striking, amounting to an increase of 21.2%. In both the obese and post-obese subjects, however, the rise in RMR was only half that seen in the lean subjects, that is, 9.6% (Table 1). This difference in thermogenesis was seen despite the fact that similar rises in plasma noradrenaline were achieved in the three groups. Although the RMR in the lean group during the infusion was still less than that in the obese patients, similar metabolic rates were found during infusion in the lean and post-obese groups. The high pre-infusion RMR of the obese group was consistent with similar measurements made on other days and was therefore not due to stress but was related to their increased lean body mass⁸. The plasma adrenaline levels remained unchanged during the basal period and were similar in all three groups. There was also no increase in plasma adrenaline during the infusion, again indicating that stress played no part in the thermogenic differences.

During the infusion there was no significant difference between the groups in the rise of either the free fatty acids (FFA) or glucose (Table 1). However, the obese group showed a greater rise in the plasma glycerol and a marked increase in the insulin/glucose ratio, indicative of insulin resistance. As changes in plasma glycerol are considered a better index of changes in lipolysis than FFA responses, these results suggest that the lipolytic response to noradrenaline was greater in the obese. The

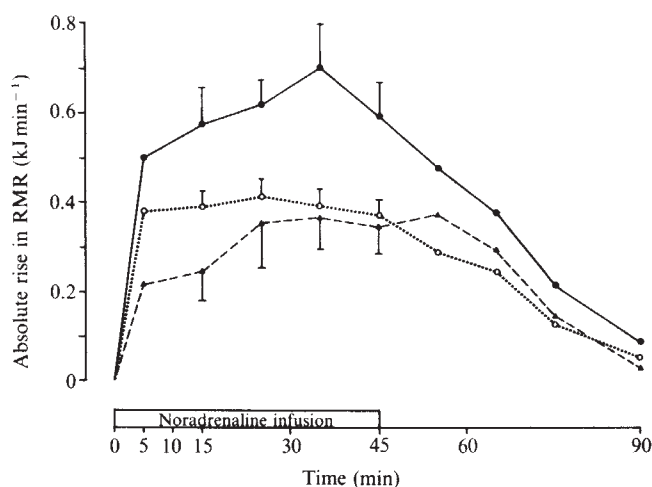


Fig. 1 Absolute increase in RMR of women during and after a 45 min intravenous infusion of noradrenaline. ●, Lean; ○, obese; ▲, previously obese, now lean. RMR was measured by the ventilated hood technique using a Servo paramagnetic oxygen analyser and IR carbon dioxide analysis²⁰. RMR measured by this technique agrees with direct calorimetry with a difference of 0.09%⁸. Values are means $\pm$ s.e.m. Fully informed consent for the procedures was obtained and ethical approval was given by the unit's ethical committee.

Table 1 Basal and peak hormone and substrate concentrations during noradrenaline infusion

Subjects	Weight (kg)	Mean% of ideal body weight ¹⁷	Noradrenaline (ng ml ⁻¹)		Free fatty acid (μmol l ⁻¹)		Glycerol (μmol l ⁻¹)		Resting metabolic rate (kJ min ⁻¹)		Insulin to glucose ratio	
			Basal	Absolute rise	Basal	Absolute rise	Basal	Absolute rise	Basal	Absolute rise	Basal	At peak† insulin response
Lean (7)	49.5	-11	0.298	1.015	622	796	57.4	89.6	3.326	0.705	2.05	2.90
	±		±	±	±	±	±	±	±	±	±	±
	2.0		0.052	0.222	31	79	3.9	14.6	0.102	0.104	0.18	0.39
Obese (6)	84.1	+53.3	0.281	1.225	712	962	78.5*	152.7**	4.309***	0.415*	3.20*	4.30*
	±		±	±	±	±	±	±	±	±	±	±
	5.6		0.057	0.178	100	56	7.9	16.8	0.207	0.042	0.48	0.37
Post-obese (6)	65.3	+15.6	0.230	0.812	579	947	80.3	135.8	3.849*	0.371*	2.15	2.76
	±		±	±	±	±	±	±	±	±	±	±
	2.7		0.055	0.149	81	97	11.0	21.7	0.174	0.065	0.37	0.48

Number of subjects given in parenthesis. Values are means ± s.e.m. Insulin to glucose ratio = insulin IU l⁻¹/glucose mmol l⁻¹. Significance values represent comparison of obese or post-obese with lean.

* = $P < 0.05$; ** = $P < 0.02$; *** $P < 0.01$.

† Glucose rises during infusion and falls once infusion is stopped. However, insulin release is initially inhibited by the noradrenaline infusion but rises once the infusion is stopped—hence the ratio is measured again at the peak of the insulin response about 15 min after the infusion is stopped. Noradrenaline is measured by radioenzymatic assay¹⁸, FFA by titration¹⁹, glucose using a Beckman Autoanalyser, glycerol by an enzymatic assay system (Boehringer Mannheim no. 125032) and insulin by radioimmunoassay (Amersham kit no. 1M78).

defective heat production in the obese therefore cannot be explained by a subnormal rate of lipolysis. The equivalent FFA concentrations in all three groups also suggest that substrate availability for energy metabolism was not a factor in the different rates of thermogenesis. Issekutz *et al.*⁹ have shown that the oxidation of FFA depends on the plasma concentration and that the relationship between plasma concentration and the rate of oxidation was the same in lean and obese subjects. A thermogenic system directly responsive to catecholamines therefore seems to be involved. The noradrenaline concentrations at the site of thermogenesis cannot be ascertained from this study but the markedly increased and equivalent venous noradrenaline levels in the three groups during the infusion and the similar metabolic response to noradrenaline found in this study compared with that observed previously in cold-adapted man¹⁰ infused with noradrenaline at 150% of the rate used in our study, suggest that we were close to if not at the limits of the non-shivering thermogenic capacity of all three groups.

The difference between the lean and obese groups in the thermogenic action of noradrenaline is strikingly similar to that seen in genetically obese rodents with their defects in thermoregulatory thermogenesis.⁶ In adult rats it is now evident that the process of non-shivering thermogenesis is dependent on the continued capacity of brown fat in the thoracic, cervical and subscapular areas to generate heat by mechanisms responsive to noradrenaline¹¹. The metabolic activity of brown fat when stimulated is far in excess of that expected on a weight basis. Abnormalities of mitochondrial function have recently been reported in the brown fat of *ob/ob* mice¹², but these abnormalities may be secondary to changes in the hypothalamus¹³ as the thermoregulatory system of these animals is controlled so that body temperature is maintained approximately 2 °C below their lean littermates throughout the diurnal temperature cycle and at environmental temperatures varying from 10 to 25 °C (ref. 5). Below 10 °C, however, the *ob/ob* mouse becomes very hypothermic.

Our studies suggest that a similar mechanism may be involved in adult human subjects, as brown fat has been identified even in elderly men and women¹⁴. The defective thermogenic response of the obese women conforms with the reports of their enhanced susceptibility to body cooling^{15,16} despite the additional insulation of subcutaneous fat. The defect in the women seems to be unrelated to energy intake, as two obese women who were deliberately fed 40 kcal per kg IBW per day for 7 days before testing responded to noradrenaline in a similar manner to that

after 3 weeks on a diet providing 9.2 kcal per kg IBW per day. Although an adaptive increase in the metabolic response to noradrenaline in man has been reported, the effect was observed only after exposure to prolonged and severe cold¹⁰.

We suggest, therefore, that the thermogenic abnormality in the obese women is constitutive rather than the result either of developmental or other environmental factors. If a direct relationship can be established between the thermogenic defect and metabolic efficiency in human subjects as well as in animals, then this test could prove to be a useful method for assessing the propensity to develop obesity in man.

We thank Mrs T. Saich and Mr G. Jennings for technical assistance and Dr Paul Trayhurn for helpful discussions. P.S.S. is in receipt of a Commonwealth Research Scholarship.

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Territorial behaviour of *Pemphigus* gall aphids

ALTHOUGH insects are known to defend nests, breeding sites and females¹, the defence of feeding sites is less well documented. Other than the defence of egg clutches and nymphs by female treehoppers² and the existence of a soldier caste in woolly aphids³, territoriality has not been reported in the large insect order Homoptera which includes aphids, scale insects, hoppers, cicadas and whiteflies. As the evolution of territoriality is thought to be directly correlated with competition for resources in short supply⁴, territorial behaviour should only be exhibited when population densities approach the carrying capacity of the environment. Because parthenogenetic reproduction and the high population growth rates of aphids seem contradictory to the notion of limited resources, aphid territorial behaviour is not expected. I report here on the settling behaviour of the aphid, *Pemphigus betae* Doane, which forms galls of the leaf blade of narrowleaf cottonwood, *Populus angustifolia*. We have quantified the existence of a defended micro-territory, the production of a floater population of individuals displaced through competitive interactions, and the differential mortality of residents and floaters which favours the evolution of territorial behaviour.

Field observations and experiments were carried out in the springs of 1976 and 1978 near Ogden, Utah. As leaf buds begin to break in early spring, colonising stem mothers of *P. betae* emerge from overwintering eggs and migrate from the base of the tree to developing leaves. First instar stem mothers are black, about 0.6 mm long, and although wingless, are highly mobile. Within 4 or 5 days of arrival, most have settled and begun to methodically probe immature leaf tissues with their stylets to induce gall formation. Each stem mother is soon enclosed within a hollow gall where up to 300 progeny are produced parthenogenetically. Survival, number of progeny, and other measures of relative fitness are correlated with gall position on the leaf blade and mature leaf size^{5,6}. When two or more stem mothers occupy the same leaf, they arrange themselves linearly along the midrib. Stem mothers occupying the base of large leaves achieve the greatest success, and these gall sites are highly preferred^{5,6}.

When competitor densities exceed the number of superior gall sites available, territorial interactions occur during the brief colonisation phase before stem mothers become physically isolated by expanding leaf gall tissue. The outcome of these interactions is determined by kicking and shoving contests (Fig. 1) in which the largest stem mother usually wins or successfully defends a linear territory of about 3 mm at the base of the leaf blade. Body size was conservatively measured as the width of the semi-sclerotised prothorax. From microscope measurements of 54 pairs of first-instar competing stem mothers, the average prothorax width of basal stem mothers was 6.3% greater than that of their distal competitors (two-tailed paired *t* test, $t = 3.298$, $P < 0.005$). In agreement with these findings, Whitham⁶ demonstrated that the removal of the defending basal stem mother can result in the downward shift of the remaining stem mother to occupy the superior basal position.

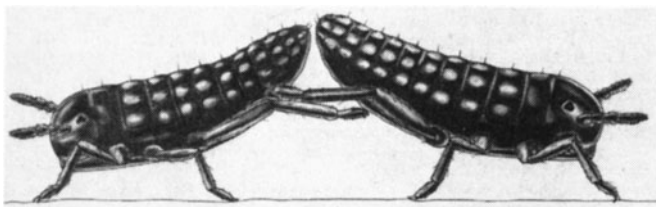


Fig. 1 Typical back-to-back fighting posture of two competing stem mothers. The largest stem mother usually wins the superior basal position. Drawing by Pam Lungé.

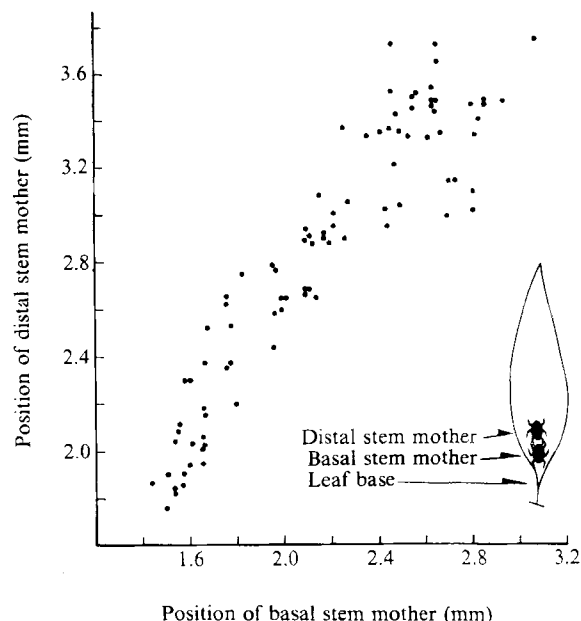


Fig. 2 Results of a 2-d contest in which the position of the subdominant distal stem mother is plotted as a function of the position of the dominant basal stem mother. Linear regression analysis ($r = 0.934$, $n = 88$, $P < 0.001$, $y = 1.17x + 0.28$) demonstrates that during contests, the position of each stem mother is based on the position of its competitor. Because stem mothers linearly arrange themselves along the midrib of the leaf blade, a single measure obtained with a caliper (the distance from the centre of each stem mother to a point marked at the base of the leaf) accurately quantifies position.

Contests can last 2 days and the basal stem mother can be displaced from her position. Figure 2 shows results from such a contest which began when a second stem mother arrived at an occupied leaf and displaced the resident to a suboptimal distal position. Positions were recorded every 10–15 min during daylight hours. During 44 of 88 recordings made, stem mothers were engaged in kicking and shoving contests in which no feeding or gall-forming activities occurred. The position of the subdominant distal stem mother on the leaf blade is plotted as a function of the position of the dominant basal stem mother. The high degree of correlation ($r = 0.934$, $P < 0.001$) indicates that stem mothers move like boxers in a ring, continually sparring to determine the winner. On the third day, only one stem mother remained. This example of an extended contest is unusually long for insects. Most observations of territorial contests have shown that the outcome is determined within a few minutes^{7–9}. If contest time is evolutionarily determined, then contests lasting more than a few minutes suggest that great dividends or losses are at stake¹⁰.

Territorial interactions can produce a floater population of stem mothers searching for places to settle. Two adjacent trees differing greatly in the densities of competing stem mothers were selected for removal experiments. On one arm of selected Y-shaped twigs on both trees, all arriving stem mothers were removed every day for a period of 7 days; the control arm of each twig was sampled only on day 7. It was expected that on the high density tree where competitive pressures for limiting gall sites should be greatest, individuals of inferior competitive abilities would accumulate on removal twigs due to competitive release. In this case, the sum of all stem mothers removed would be greater than the number of stem mothers on control twigs. In comparison, on the low density tree, competitive pressures should be much reduced, enabling stem mothers to settle quickly without further movement. Consequently, few or no differences were expected in the settling patterns of stem mothers on removal or control twigs. On each tree approximately 2,500 leaves were examined. Figure 3 shows the pattern of stem

mother arrival on removal twigs. Of the 699 stem mothers attempting colonisation on the high density tree, 83% arrived over a 3-d period that peaked just after bud burst; on the low density tree, colonisation spanned the same time period but the peak was much reduced. Figure 3b, shows that when competitor densities are high, colonising stem mothers rapidly respond to the removal of competitors. The total number of stem mothers removed during seven consecutive days of sampling (535 per 1,000 leaves) was 69% greater than the number of stem mothers found on control leaves (316 per 1,000 leaves) ($\chi^2 = 46.67$, $P < 0.001$). On the tree with a low competitor density, however, the number of stem mothers collected on removal twigs (72 per 1,000 leaves) did not differ significantly from the number of stem mothers found on control leaves (61 per 1,000 leaves) ($\chi^2 = 1.11$, $P > 0.29$). The fact that there was a highly significant difference between treatment and control on the high density tree shows that stem mothers were moving in search of a place to settle and were reacting negatively to the presence of other stem mothers. On the other tree, however, densities of potential competitors were so low that competition for a limited number of gall sites was reduced, enabling all stem mothers to rapidly select leaves and immediately settle. Thus, with high population densities, many stem mothers are displaced through competitive interactions and a floater population is produced. These results are similar to those obtained in other systems as diverse as birds, mammals and fish, where subdominants or floaters moved into vacated spaces¹¹⁻¹⁷.

Experiments indicate that the floater population suffers much greater mortality than the resident population. Before all stem mothers had permanently settled during colonisation, the positions of 211 stem mothers distributed among 351 leaves of a small branch were recorded. A sticky barrier (Tanglefoot) was placed at the base of the branch to prevent further recruitment. After all stem mothers had permanently settled, their positions were again recorded. Forty stem mothers had abandoned the positions they had held during the first census and attempted gall formation on other leaves. Of these individuals, only 24% survived, while 72% of those that did not move from their original positions survived ($\chi^2 = 21.45$, $P < 0.001$). This experiment has been repeated with another *Pemphigus* species and the results were identical⁵. Such differential mortality could be an important factor in the evolution of territorial behaviour and may account for the observed extended contest of Fig. 2.

The Eriosomatidae, or gall-making aphids of which *Pemphigus* is a member, exhibit a unique reproductive trait in which each stem mother is the sole progeny of a female sexual¹⁸. As

no other aphid group or known animal lays only one egg throughout their lifespan, this trait must have evolved under rather severe and/or unusual circumstances. I suggest that the advantage large body size confers in territorial interactions may have contributed to the evolution of this trait by favouring the reduction of clutch size and the placement of all resources into a single large egg.

I thank J. Alcock, H. R. Pulliam, C. C. Smith and W. V. Zucker for comments on the manuscript and acknowledge the support of NSF grant no. DEB 7905848.

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Received 15 January; accepted 20 April 1979.

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Endomycorrhizal fungi and *Rhizobium* as biological fertilisers for *Medicago sativa* in normal cultivation

LEGUMES can form two types of symbiotic association with microorganisms. One, with *Rhizobium* sp., is involved in the fixation of atmospheric nitrogen; the other, with fungi of the family Endogonaceae that form vesicular-arbuscular (VA) endomycorrhizas, is concerned with the uptake of phosphorus by the plants. Glasshouse experiments have demonstrated that legumes inoculated with both types of microorganism grow and nodulate better, and have higher nitrogenase activity and phosphorus content than plants that are uninoculated or inoculated with either *Rhizobium* or mycorrhizal fungi separately¹⁻¹². Also, plants with both types of symbiosis may be important as pioneer colonisers of nutrient-deficient habitats¹³. At present, the possibility of field inoculation with mycorrhizas to improve yield, and the subsequent economy in the use of chemical fertilisers, are being considered¹⁴. Positive responses to VA mycorrhizas are to be expected mainly in soils low in nutrients, particularly phosphate, and where indigenous endophytes are sparse or inefficient¹⁴. Thus, the use of soil sterilisants to destroy indigenous endophytes has been assayed¹⁵⁻¹⁶. There are also reports on the effect of VA fungi inoculation in non-sterile soils¹⁷⁻²⁰. The effects of endomycorrhizas on legumes in relation to the improvement of hill¹⁹⁻²⁰ and marginal soils are now being studied²¹. But as far as we know no data for leguminous crops growing on non-sterile arable soils in standard agricultural conditions in temperate regions have been published. We report here that inoculation of *Rhizobium* and *Glomus* improves the growth and nutrition of *Medicago sativa* in normal cultivation on an arable field.

The experiment was carried out on an irrigated calcareous soil (pH, 7.8) in a fertile valley ('vega') in Granada province, Spain. Its texture was: 25.2% sand, 30.0% loam and 44.8% clay. The soil contained 1,302 p.p.m. total N, 415 p.p.m. total K,

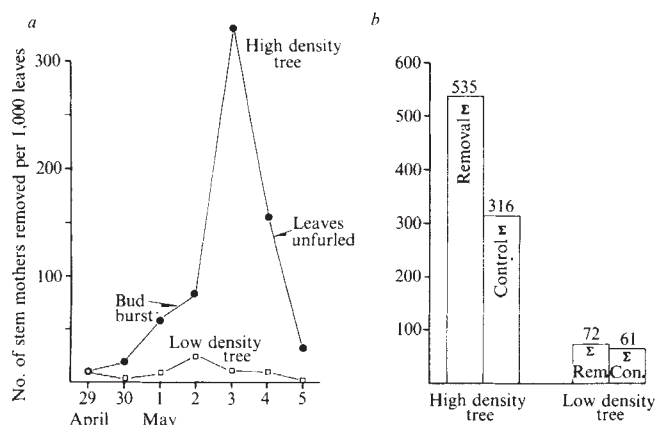


Fig. 3 Results of a removal experiment designed to test the hypothesis that due to territorial interactions occurring during leaf selection, a floater population is produced. Y-shaped twigs on two trees which differed in the density of competing stem mothers were selected. One arm of each twig acted as the control and was sampled only at the end of the experiment. On the other arm stem mothers were removed each day. *a*, Shows the number of stem mothers removed each day from both trees; *b*, compares the removal and control twigs for both trees (see text).

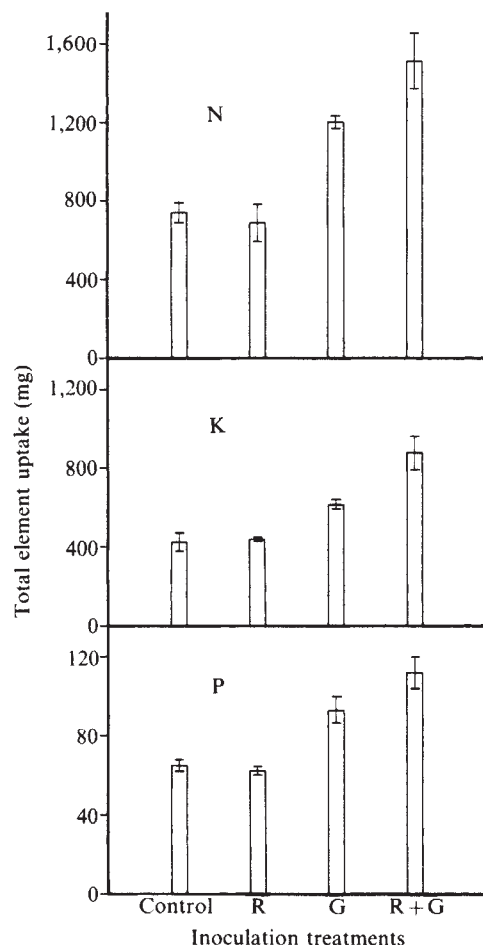


Fig. 1 Effects of *Glomus* (G) and *Rhizobium* (R) on N, P and K uptake by *Medicago sativa* growing in an arable field soil under normal cultivation. Total N, P and K taken up by the plants is calculated from data of shoot dry weights and the percentage of element (mean of four replicates). Standard errors are shown.

611 p.p.m. total P, 9.2 p.p.m. available phosphate²³ and 1.74% organic matter. This field has been intensively cultivated for centuries and two crops per year are harvested. When the present experiment was designed the field had been left fallow for six months. Endogonaceae spores were recovered by the technique of wet sieving and decanting²². The number of spores per 100 g soil was: 14 ± 1.4 yellow vacuolate²⁴ (*Glomus mosseae*²⁵), 2 ± 0 laminate²⁴ (*Glomus macrocarpus*²⁵) and 8 ± 0.7 unidentified. Spore numbers were, therefore, low but fall in the range of about 0.1–5 per g soil, recovered in most cases²⁶. The predominance of *Glomus mosseae* spores in this field would justify, from the ecological point of view, the choice of this species as inoculant.

Medicago sativa L. cv. Aragón was the host plant for an endomycorrhizal fungus of the yellow-vacuolate spore type and for *Rhizobium meliloti* 203. Four seedbeds were prepared for plants for the field experiment. These were: uninoculated control (C), *Rhizobium*-inoculated (R), *Glomus*-inoculated (G) and *Rhizobium* + *Glomus* inoculated (R+G). The seedbeds were kept for 20 d in a glasshouse at 19–25°C. At this time, seedlings had a slight VA infection; about 3–5% of their root system. The experimental field was divided into four plots: C, R, G and R+G, to each of which seedlings from the corresponding seedbed were transplanted. Each plot consisted of four replicates with each replicate containing five 1 m² microplots. These microplots received 25 groups of three plants. Care was taken in selecting uniform seedlings and allowing some soil from the seedbed to adhere to their roots. Seedlings of R and R+G treatments were reinoculated with *Rhizobium* at transplanting.

Table 1 Effects of *Glomus* and *Rhizobium* on the yield of *Medicago sativa* growing under normal cultivation in an arable field soil

Inoculation treatments	Shoot dry weight (g)
Uninoculated controls	15.18 ± 0.95
<i>Rhizobium</i>	15.94 ± 1.19
<i>Glomus</i>	22.56 ± 1.11
<i>Rhizobium</i> + <i>Glomus</i>	32.09 ± 1.77

Mean of four replicates.

Plants were irrigated by the farmers in their usual way, and no chemical fertilisers were applied during the experiment. At harvest, plants which grew during 12 weeks in the five microplots belonging to the same replicate were pooled, dry weights of shoots were recorded and analysed for P, N and K (ref. 27).

Table 1 records plant growth. It is clear that *Glomus* inoculation improved the yield of alfalfa, whereas *Rhizobium* inoculated alone did not. Uninoculated control plants showed nodulation by indigenous rhizobia, however, in *Glomus*-inoculated plants the introduction of *Rhizobium* was effective and plant dry weight was increased by nearly 50% (R+G treatment compared with G treatment). The available P content in the test soil is low; this probably not only determined the response of the plants to *Glomus* inoculation but also that plants not given a *Glomus* inoculum did not respond to *Rhizobium* inoculation. Phosphorus is known to be an essential element for N₂ fixation^{8,21} and as it was a limiting factor in this experiment, plant growth and *Rhizobium* activity were not greater than for the uninoculated controls unless the plants were adequately mycorrhizal. *Glomus* inoculation improved total N, P and K uptake and the inoculation with *Rhizobium* was only effective when applied together with *Glomus* (Fig. 1).

The main conclusion from these results is that the mycorrhizal fungi *Glomus mosseae* is an effective 'biological fertiliser' for *Medicago sativa*. Inoculation with *Glomus* not only affected plant growth and nutrition but also enhanced the activity of *Rhizobium meliloti* when it was applied as inoculant. The introduction of *Rhizobium* together with *Glomus* and the subsequent establishment of effective dual symbiotic association with alfalfa was successful, as yield was increased by 211% compared with control. This suggests that it may be possible to reduce the chemical fertiliser inputs to this arable crop. This study may have practical significance for neutral-alkaline soils like the one used in the present investigation. As this soil is under intensive cultivation it has received, and is receiving, large amounts of organic and inorganic additives; as a result, the total P is high but the available P is quite low, probably because the added P fertiliser has become fixed, mostly by the calcium in this high pH soil, or by some minerals of the clay fraction²⁸, which is relatively high in the test soil. The extensive use of fertilisers also accounts for the low number of Endogonaceae propagules²⁹.

We thank Dr D. S. Hayman for criticism and corrections, Dr B. Mosse for providing mycorrhizal fungus, Dr J. L. García-Chicano and Chemistry Department, Zaidín, for some chemical analyses.

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Received 15 January; accepted 6 March 1979

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Anomalous temperature dependence of the sodium conductance in rabbit nerve compared with frog nerve

THE sodium channel in excitable tissues is an integral component of the membrane, and is intimately associated with the surrounding lipid¹. Thus, the electrophysiological parameters of the sodium channel might be expected to exhibit a characteristic dependence on temperature, reflecting the marked temperature sensitivity of the physical properties of most lipids. We report here voltage-clamp experiments which show that cooling a rabbit node below the region of about 6 °C does in fact sharply decrease the maximal sodium conductance, and markedly prolongs sodium inactivation. It thus seems that the lipid (or lipid-protein) environment of the sodium channel in the rabbit node undergoes a drastic change below 6 °C.

Single myelinated fibres from rabbit and frog (*Rana pipiens*) sciatic nerves were dissected and voltage-clamped². The nerve chamber was initially allowed to stabilise at room temperature (25 °C). Pre-cooled liquid was then pumped through the brass block enclosing the nerve chamber, gradually cooling the whole preparation to about 0 °C in 15 min. The temperature was measured throughout the experiment by a small thermocouple (50 µm diameter) located about 1 mm beneath the node in the pool containing the node. A single fixed depolarisation to -20 mV (rabbit) or to -5 mV (frog), preceded by a hyperpolarising prepulse to -125 mV to remove sodium inactivation, was used to assay sodium conductance every 0.3–1 °C as the temperature fell from 25 to 0 °C. At the end of the experiment, the temperature dependence of the internodal resistance was measured over the same temperature range and used to correct for consequent changes in current. Typically, the internodal resistance increased progressively, rising by a factor of about 1.3 in both frog and rabbit nerve when the temperature fell by 10 °C. The net ionic current associated with the test pulse in a rabbit node consisted of an inward current superimposed on an outward leak current, potassium current being virtually absent in the mammalian node^{2,3}. In frog nerve, tetraethyl-ammonium was added to block potassium current. After subtracting the leak component from the total ionic current, the values of τ_h for both frog and rabbit nodes were determined by fitting the sodium current to the expression:

$$I_{Na} = G_1 e^{-t/\tau_h} (1 - e^{-t/\tau_m})^p$$

with $p = 2$ for rabbit and $p = 3$ for frog.

Figure 1 shows typical sodium current records from a frog and rabbit node of Ranvier at different temperatures. A 5 °C fall in temperature from 23 °C slowed the kinetics of the sodium

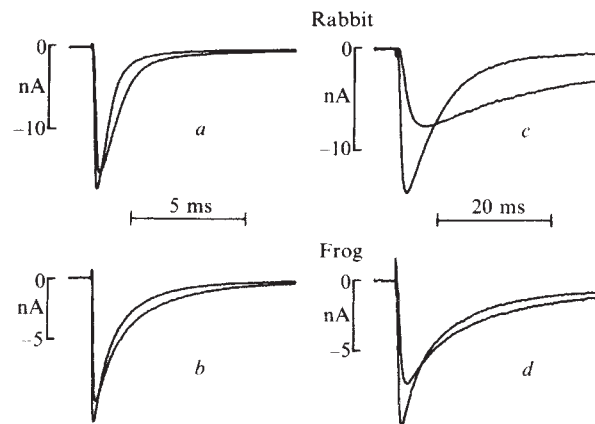


Fig. 1 Sodium currents in a rabbit (a, c) and frog (b, d) node. Each pair of currents shows the response at a given temperature (23 or 6 °C) superimposed on one at a temperature 5 °C lower: a and b, 23–18 °C; c and d, 6–1 °C. In the rabbit E_{Na} was +70 mV at 25 °C and +65 mV at 0 °C; in the frog the corresponding values were +58 and +65 mV, respectively. The sodium current after leak subtraction and correction for temperature-dependent changes in the internodal resistance was calibrated by assuming a value of 10 mΩ for the internodal resistance at 25 °C. Note that the time to peak is increased relatively more in c than in a.

current in both frog and rabbit nerve by about the same extent and produced a similar decrease in the size of the current (Fig. 1a, b). However, a 5 °C fall in temperature from 6 °C produced a much bigger decrease in the peak current and a larger prolongation of sodium inactivation in the rabbit than in the frog (Fig. 1c, d). These effects of cooling were reversible on re-warming.

Figure 2 shows in greater detail the temperature dependence of the maximal sodium conductance, $\bar{g}_{Na}$, and the time constant of inactivation, τ_h , for five rabbit and six frog nodes. The value of $\bar{g}_{Na}$ was determined by extrapolating the decay phase of the sodium current back to the onset of the test pulse at each temperature. Figure 2 shows clearly that the curves for the temperature dependence of these parameters in frog and rabbit nodes have different shapes; in the frog the slope of the curve remains roughly constant over the whole temperature range^{4,5}, whereas in the rabbit there is a marked change in slope at about 6 °C. Thus, the value of τ_h for rabbit at -20 mV increased by a factor of about 1.73 for a 5 °C drop in temperature from above 15 °C (Q_{10} about 3), whereas below a transition temperature region (about 6 °C) the same drop in temperature increased τ_h 5.7-fold (Q_{10} about 33). Similarly, the values for $\bar{g}_{Na}$, which depended only moderately on temperature above 6 °C (Q_{10} about 1.7), became much more sensitive to temperature below 6 °C, the Q_{10} for the decrease being about 4.7. Because the rising phase of the sodium current was relatively fast, and because relatively few computer sampling points were taken during it, measurements of τ_m were too uncertain for us to determine whether or not a similar discontinuity occurred with this parameter.

Several factors besides a reversible reduction of $\bar{g}_{Na}$ could have caused the marked decrease of sodium current at low temperatures: for example, a reduction in E_{Na} or shifts of the $h_{\infty}(E)$ and $P_{Na}(E)$ curves with temperature. In the present study, the average values of E_{Na} at 25 °C and 0 °C were both 65 mV for frog nodes, and 65 mV and 60 mV, respectively, for rabbit nodes. A decrease of as much as 10 mV on cooling would have decreased the sodium current associated with the test pulse (at -20 or -5 mV) 1.2-fold at most. Furthermore, although the $h_{\infty}(E)$ curves in both frog and rabbit were consistently shifted in the hyperpolarising direction (by 5–19 mV) when the temperature was lowered from 25 °C to 0 °C, this shift could not have contributed significantly to the observed decrease in sodium current because a large negative prepulse was used to remove any inactivation. Finally, any shift in the $P_{Na}(E)$ curve was small

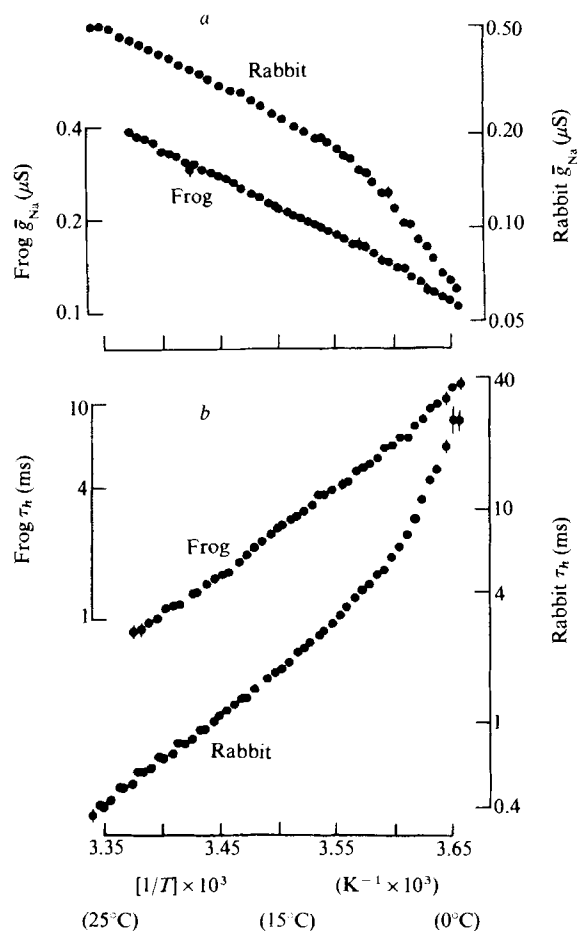


Fig. 2 Arrhenius plot of maximal sodium conductance $\bar{g}_{Na}$ (a) and of time constant of inactivation τ_h (b) for frog and rabbit nodes. The data obtained from five rabbit and six frog nodes were normalised in each experiment by finding a least squares fit to the data points between 18 and 12°C and scaling all experimental points for that node so that the conductance at the mid-points of the lines (the expected value at 15°C) were 0.27 μS and 0.25 μS for the rabbit and the frog nodes, respectively, in panel a, and the corresponding times were 1.4 ms and 1.9 ms for the rabbit and the frog, respectively, in panel b. These values were chosen because they were the actual average values for $\bar{g}_{Na}$ and τ_h obtained experimentally. Each point is the average of 2–9 observations for $\bar{g}_{Na}$ and 2–19 observations for τ_h . The error bars drawn through each point (± 1 s.e.m.) are usually smaller than the symbols. The rate of fibre rundown was slow compared with the rate of cooling and was not corrected for. However, rewarming took about 5 times as long leading to a hysteresis in the $\bar{g}_{Na}$ but not the τ_h curves. Two rewarming experiments are included in the mammalian τ_h curve.

or absent ($< \pm 10$ mV) and so could not have caused any substantial variation in current size with temperature since the test pulse of 60–75 mV was in the potential range in which the $P_{Na}(E)$ curve had already reached a plateau, at both 25°C and 0°C.

The temperature-dependent transition reported here for the conductance of the sodium channel of mammalian myelinated nerve thus resembles the temperature-dependent transition already reported for the acetylcholine-activated channel conductance^{6,7} and for other membrane proteins where activity, transport or mobility within the lipid have been assayed as a function of temperature¹; it also resembles the transition found in frog nerve and muscle⁸. A change in membrane fluidity is thought to occur at these transition temperatures. It is possible that the same mechanism underlies the marked temperature dependence of the various functional parameters of sodium channel reported here for the rabbit node of Ranvier. Alternatively, a conformational change in the channel protein might

have occurred. The fact that there seems to be little or no discontinuity in the electrical parameters of the frog node of Ranvier in the present study may reflect species differences in the lipid composition of the membrane, or differences in the sodium channels themselves. Interestingly, in the rabbit nerve the transition temperature region is about the same for τ_h and $\bar{g}_{Na}$, suggesting that the molecular structures regulating $\bar{g}_{Na}$, and those controlling the gating process, are surrounded by a similar lipid environment⁶. The nature of the marked reduction of $\bar{g}_{Na}$ at low temperature is unclear; it may result either from a temperature-dependent block of the sodium channels or from a reduction in the single channel conductance. These alternatives might be distinguished by noise analysis experiments. If the transition temperature reported here is related to a phase change in the lipid surrounding the channel, then it might be possible to alter this temperature using agents that modify membrane fluidity.

We thank Dr J. V. Howarth for help in the construction and mounting of the thermocouple used in these experiments. We also thank Dr W. Schwartz who introduced us to the method of cooling. This work was supported in part by PHS grant NS 12327 and grant RG 981-A-1 from the US National Multiple Sclerosis Society.

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In vitro activation of a human macrophage-like cell line

SEVERAL permanent murine macrophage-like cell lines exhibiting various macrophage-associated effector functions have been described^{1–3}, but establishment of permanent human macrophage cell lines has been much more difficult⁴. Recently Sundström & Nilsson⁵ reported the establishment of a human histiocytic lymphoma cell line, U937, with macrophage characteristics. This line was further adapted to rapid growth *in vitro* by Lachman *et al.*⁶. During studies of major histocompatibility complex antigens on a number of human lymphoid cell lines we failed to detect Ia-like antigens (HLA-DR) on U937. Because these alloantigens have been reported to occur on early myeloid cells⁷ and might therefore be regarded as differentiation antigens of stem cells, an attempt was made to produce differentiation and expression of new cell surface molecules on U937. Procedures similar to those used to produce differentiation of myeloid cell lines⁸ and promote growth of macrophages in culture were tried. Although U937 cells failed to express Ia-like antigens they underwent remarkable morphological and functional changes. We report here that U937 can be activated by supernatants from human mixed lymphocyte cultures (MLC). Although this activation takes several forms, the findings reported here show marked augmentation of antibody-dependent cellular cytotoxicity (ADCC) against erythroid and tumour target cells.

Figure 1 presents photomicrographs of normal U937 (Fig. 1a) and activated U937 cells (Fig. 1b). Activation markedly alters

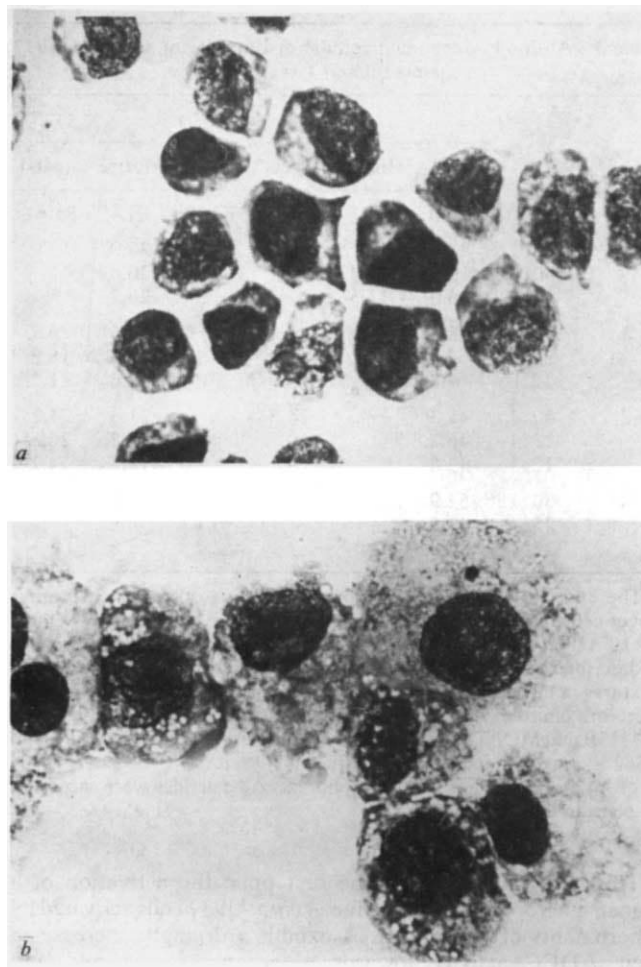


Fig. 1 Cytochrome preparations of unactivated (a) and activated (b) U937 cells. Human macrophage-like line U937 was adapted to continuous growth in suspension culture independent of monolayer cells or their supernatants⁵. Generation time was 16–20 h. Unactivated cells (a) are grown in RPMI-1640 supplemented with 10% FCS. Activated cells (b) were cultured for 20 h in normal culture medium supplemented with 30% conditioned medium obtained in a one-way MLC on day 6 of culture. The CM used for the experiments presented in this paper used peripheral mononuclear cells from donor DS and an allogeneic B-cell line JoKo (irradiated) as stimulator. Wright stain, $\times 672$ magnification.

the appearance of U937 cells: the cells become larger, the cytoplasm more vacuolated and the membrane more villous. In culture the activated cells are more adherent and have increased intensity of nonspecific esterase staining (not shown).

One of the principal functions of macrophages is their ability to mediate ADCC^{1–3}. We therefore chose the ADCC assay to measure differences in activity of U937 macrophage-like cells. The ADCC data of five representative experiments are presented in Table 1. Unactivated U937 cells exhibited varying levels of ADCC activity against chicken red cell targets modified with TNP (CRC-TNP), as represented in the minimal levels of activity in experiments 1, 2, 4 and 5a, with higher levels in experiment 3. The reason for the fluctuating baseline ADCC levels of normal unactivated U937 is not clear. This variability may be caused by cell-cycle dependence of activity or 'nonspecific' activation by fetal calf serum (FCS) components. In contrast, U937 cultured with supernatants of human MLCs (conditioned medium, CM; see legend to Table 1), consistently showed increased activity in ADCC against CRC-TNP targets. This strong activity of activated U937 was not restricted to CRC-TNP targets; similar results were observed using sheep red cells modified with TNP (SRC-TNP) (Table 1, experiment 5b). Neither activated nor unactivated U937 were cytotoxic against

Table 1 Antibody-dependent cellular cytotoxicity of activated U937 cell line against erythroid target cells

Expt	Activating* supernatant	ADCC/CRC-TNP % Specific lysis			
		8:1	4:1	2:1	1:1
1	None	8.5	5.4	2.5	1.3
	CM	57.1	47.1	33.8	18.7
2	None	4.1	1.7	0.8	–0.8
	CM	56.5	54.1	46.8	29.6
3	None		21.4	11.9	8.5
	CM		54.2	42.2	31.9
4	None	1.9	0.8	0.0	0.1
	CM	31.8	19.7	11.7	5.8
	JoKo†	4.4	2.4	2.6	0.8
	DS‡	6.6	4.2	1.9	0.8
5a	None	1.3	0.9	0.5	0.5
	CM	41.1	33.2	24.5	15.2
5b		ADCC/SRC-TNP			
		8:1	4:1	2:1	1:1
5b	None	5.1	1.3	1.6	–0.5
	CM	18.9	12.2	10.9	11.4

The effect of U937 activation on ADCC against erythroid target cells. ADCC was tested in a 2-h ⁵¹Cr release assay against 3×10^4 chicken red cells (CRC) or sheep red cells (SRC) modified with 2,4,6-trinitrobenzene sulphonic acid (TNP) and coated with rabbit anti-TNP antibodies, a method previously described¹. The ⁵¹Cr released in such an assay was due only to extracellular killing². Assays were carried out in triplicate and s.e. did not exceed $\pm 5\%$. Spontaneous release of the targets in medium alone ranged between 0.5 and 2.0%. Since insignificant ADCC activity was shown by unactivated or activated U937 cells in the absence of anti-TNP antibodies, the results presented here show only the killing in the presence of anti-TNP antibodies.

* Conditioned medium (CM) was obtained as described in Fig. 1.

† CM from irradiated JoKo (B-cell line) cultured alone.

‡ CM from peripheral mononuclear cells of individual DS cultured alone.

CRC-TNP or SRC-TNP in the absence of sensitising antibodies (data not shown).

To assess the extent to which product(s) of MLC supernatants were responsible for U937 activation, control experiments using supernatants of the responder cells or the irradiated stimulator cells of the MLC grown alone, were performed. Culturing U937 with CM from culture of the responder alone (experiment 4—DS) or irradiated stimulator cells of the MLC (experiment 4—JoKo) resulted in low ADCC activity similar to that by unactivated cells (experiment 4).

The possibility that CM could enhance ADCC if present in the assay by a carry-over mechanism was also tested. CM added to

Table 2 Phagocytosis of erythroid target cells by activated U937

Activating supernatant	Anti-TNP	% Phagocytosis				
		Expt 1	Expt 2	Expt 3	Expt 5a	Expt 5b
None	–	8.3	10.3	6.6	–0.8	–3.2
None	+	12.8	12.2	25.7	0.6	3.4
CM	–	7.9	10.5	7.7	–0.9	–0.8
CM	+	17.9	29.1	26.1	10.2	39.9

The effect of U937 activation on phagocytosis of erythroid target cells. Phagocytosis was determined by measuring ⁵¹Cr of the endocytosed erythrocytes (CRC-TNP and SRC-TNP) by U937, according to the method of Walker and Demus². The phagocytosis experiments presented here correspond to the ADCC experiments with the same number as shown in Table 1. Therefore, experiments 1, 2, 3 and 5a were done with CRC-TNP, whereas SRC-TNP were used for experiments 5b. The E:T ratio chosen for the phagocytosis experiments was 8:1. Groups without anti-TNP antibodies added represent Fc-independent phagocytosis, whereas groups with anti-TNP antibodies reflect Fc-dependent phagocytosis ('immune phagocytosis').

unactivated U937 cells during the 2-h ADCC assay, however, did not alter their activity (data not shown).

The activation of U937 is rapid. Enhanced ADCC against CRC-TNP was seen 6 h after addition of CM and reached a twofold increase after 28 h (Fig. 2a). In another study stimulation was measured as early as 3 h, reaching a peak after 3–5 d. Cultures fed with normal medium gradually lost activity after 3 weeks.

As ADCC is Fc receptor (FcR) dependent it was of interest to test whether activation of U937 correlated with FcR expression by these cells. A steady increase in the number of FcR+ cells was indeed detected by rosetting with antibody-coated erythrocytes (EA rosettes) as shown in Fig. 2b. A doubling in the number of FcR+ cells occurred during the first 28 h. By using aggregated ^{125}I -IgG, as an independent measure of FcRs, a similar rising pattern was seen during activation. These data (not shown) clearly establish a direct relationship between the number of FcR+ cells in the activated U937 population and Fc-dependent phenomena such as ADCC.

Phagocytosis by activated U937 was enhanced in several experiments (Table 2, experiments 2, 5a and 5b) while in others (experiments 1 and 3) it did not exceed phagocytic levels of the unactivated U937. In all cases a greater percentage of target cells was phagocytosed when antibodies were present (antibody-dependent) than when they were absent (antibody-independent).

Although human peripheral blood monocytes have repeatedly been shown to mediate ADCC to several red cell targets⁹, their role in ADCC against tumour targets is controversial. Many studies suggest that antibody-coated tumour targets are killed by K cells^{10,11}, whereas others ascribe this effector function to both K cells and macrophages^{12–14}. Unactivated U937 cells have no ADCC activity against neoplastic cells. However, *in vitro* activation of the cell line as described in Fig. 1 resulted in significant and reproducible cytolysis of a variety of neoplastic cells (see Table 3). A wide spectrum of lymphoblastoid cell lines (LCL) and a myeloid line were lysed by activated U937 in a 4–6.5 h assay at effector:target ratios varying from 12.5:1 to 100:1. Target cells of human (HSB, MOLT4, SB and K562) and murine (EL4 and RL δ 1) origin with different surface characteristics were found to be sensitive targets.

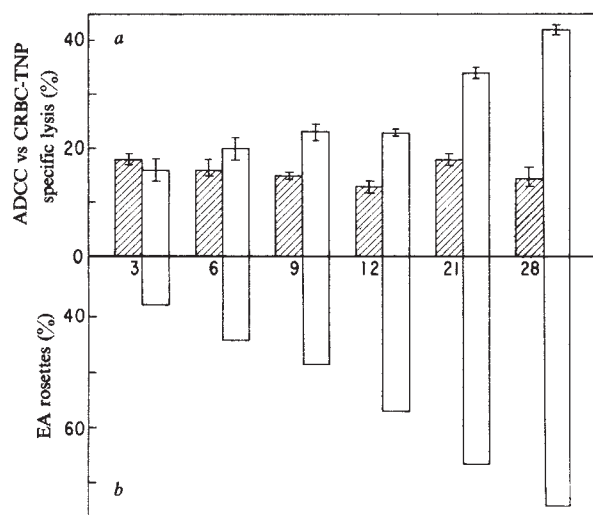


Fig. 2 Time kinetics of U937 activation and its effect on ADCC and FcR expression. *a*, ADCC of unactivated $\square$ and activated $\square$ U937 was measured in a 2-h ^{51}Cr release assay against CRC-TNP (see Table 1). U937 cell line was activated with 30% CM and samples were tested after 3, 6, 9, 12, 21 and 28 h of cultivation. *b*, FcR+ cells in the activated U937 population $\square$ were measured by their ability to form rosettes with ox red cells (ORC) sensitised with rabbit 7S anti-ORC antibodies. FcRs were expressed in 30–40% of unactivated U937 cells (data not shown).

Table 3 Antibody-dependent cellular cytotoxicity of activated U937 against tumour target cells

Expt	E:T ratio	% Specific lysis					
		Human targets*			Murine targets†		
		HSB	MOLT4	SB	K562	EL4	RL δ 1
1	100:1	22.6	13.7		4.8	29.9	
	50:1	19.0	18.4		5.3	26.0	
	25:1	16.0	13.5		6.3	26.7	
2	50:1	8.0		7.0		6.5	26.7
	25:1	5.9		3.0		5.4	18.8
	12:1	4.8		4.0		2.6	11.1
3	50:1	45.3		14.5		21.4	24.2
	25:1	46.2		10.5		17.2	26.8
	12:1	33.8		9.1		15.6	14.2
4	50:1	52.9			7.5	40.5	
	25:1	43.8			6.1	36.8	
	12:1	37.8			2.2	25.8	

The effect of U937 activation on ADCC against nucleated tumour target cells. ADCC was tested in a 4–6.5 h ^{51}Cr release assay against 1×10^4 target cells TNP-modified and coated with anti-TNP antibodies as described above (see Table 1). Controls of unactivated U937 and cell mixtures in the absence of anti-TNP antibodies were negative and were therefore omitted. Activation was performed as described in Fig. 1.

* HSB and MOLT 4 are leukaemic T-cell lines, SB is a B-cell line, and K562 is a myeloid leukaemia cell line.

† EL4 and RL δ 1 are T-cell lymphomas. All cell lines were carried as suspension cultures *in vitro*.

This study describes for the first time, the activation of a human macrophage-like cell line *in vitro*. U937 cells activated by supernatants of human MLCs exhibit a dramatic increase in their ADCC activity towards a variety of erythroid and nucleated-neoplastic targets. The activation is rapid and the marked morphological changes which occur are accompanied by an increase of FcR-bearing U937 macrophage-like cells. These results are in accordance with those of Shaw *et al.*¹³ who have demonstrated ADCC activity by peripheral monocytes against nucleated target cells although McDonald *et al.*¹⁰ and Nelson *et al.*¹¹ have reported activity mediated exclusively by K cells in their systems. The demonstration that a human macrophage-like cell line mediates ADCC against erythroid and neoplastic cells, and that this activity can be augmented, or in most cases induced in culture, will allow further investigation into the mechanism of activation and cytolysis by human macrophages. Biochemical studies of the activating molecule(s) are in progress.

We thank Dr D. B. Amos for his support, Drs A. Berger and S. Livnat for a critical review and Ms J. Kerber for typing and preparing the manuscript. This work was supported by NIH training grant GM-07171 (to J.W.L.) and PHS grants AI-08897, CA-14236-04 and CA23354-01.

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Specific depletion of alloreactive cytotoxic lymphocyte precursors

THE major barrier to replacement therapy in various clinical immunodeficiency states is the likelihood that the transferred immunocompetent T lymphocytes would react against alloantigens of the recipient and produce graft-versus-host (GVH) disease¹. The logical resolution of this problem would be to delete or inactivate those clones of T cells which are specifically coded to react against the histocompatibility antigens of the recipient tissues. The remaining cell population could then fully reconstitute the recipient without danger of GVH reactions. Specific clonal deletion of this kind has been obtained in experimental animal situations by pre-circulation through a third-party host^{2,3} or by induction of specific anti-idiotypic immunity⁴. These studies show the validity of this approach but are technically complicated and have limited application. A simpler method of obtaining this result has been sought based on the following strategy. To reconstitute a strain A mouse by transfer of lymphocytes from an allogeneic strain B, the strain B lymphocytes are first incubated on a monolayer of cells of strain A. Those clones which recognise the strain A alloantigens should adhere to the monolayer; the non-adherent populations of cells should now be unable to react against alloantigens of strain A but should still respond to alloantigens of other haplotypes. Although this kind of technique has been widely effective in removing fully activated cytotoxic lymphocytes (T_c)⁵⁻⁹, its use to deplete alloreactive T_c precursors has given variable results^{8,10-16}. Significant depletion of precursors has been reported primarily when the non-adherent cells have been assayed *in vivo*¹³⁻¹⁶, but this has been claimed to be due to change in the responder cells, not depletion, as the same population assayed by *in vitro* generation of T_c shows no evidence of depletion¹⁶. We present here a simple depletion method developed from our finding that when splenic lymphocytes are incubated for a few hours before being formed into a monolayer, they can effectively absorb alloreactive T_c precursors even when assayed by the sensitive *in vitro* assays.

Figure 1 shows the results of experiments in which BALB/c responder cells were absorbed on monolayers of spleen cells constructed by attachment to Petri dishes coated with poly-L-lysine (PLL); the non-adherent responder cells were then

cultured with X-irradiated stimulator cells and after 5 d assayed for the presence of T_c . Absorption on monolayers of fresh C57BL/10 (B10) spleen cells failed to reduce the BALB/c T_c response to stimulation with B10 alloantigen (Fig. 1a), in agreement with reports of similar failures^{8,16}. In contrast, absorption on monolayers constructed with B10 spleen cells which had been preincubated at 37°C for 4 h abolished the response to B10 alloantigens (Fig. 1b). The specificity of the absorption is shown by the ability of the same population of non-adherent BALB/c cells to mount a normal T_c response to B10.BR alloantigens, and further by the failure to deplete the response to B10 or B10.BR alloantigens when the absorbing monolayer was composed of preincubated BALB/c spleen cells (Fig. 1c). The latter experiment also demonstrates a consistent finding that absorption on a monolayer of preincubated cells commonly increases the T_c response to haplotypes other than that of the absorbing monolayer, but this is not attributable to carry-over of monolayer cells. In this experiment, and those described below, the numbers of non-adherent cells recovered after incubation on the absorbing monolayer were consistently 97-102% of the number of responder cells applied, and by immunofluorescence tests with anti-H-2 antisera, cells of monolayer haplotype comprised only 3-6% of this population.

Table 1 shows that the technique is applicable to other strains. B10.BR spleen cells were specifically depleted of T_c precursors

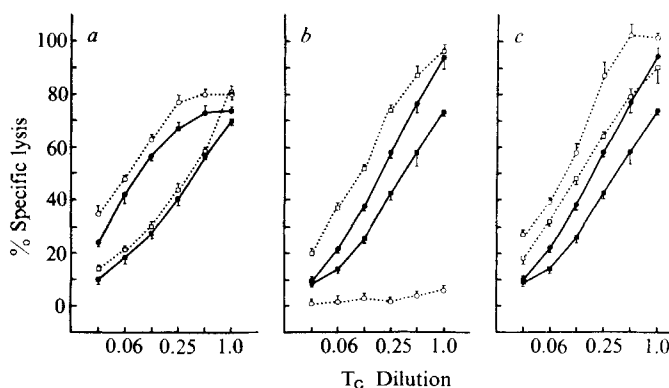


Fig. 1 Effect of pre-incubation on the ability of splenic monolayers to deplete T_c precursors. Monolayers for absorption were constructed by attaching $\sim 2 \times 10^7$ spleen cells to polystyrene Petri dishes (50×13 mm, Sterilin no. 122) previously coated with poly-L-lysine (Sigma)*. The spleen cells, prepared as previously described¹⁷, were used immediately or after 4 h incubation in 75-cm² flasks (Falcon no. 3024) containing 30 ml Eagle's minimum essential medium plus non-essential amino acids, supplemented with 10% v/v fetal calf serum (FCS) and 7×10^{-10} M 2-mercaptoethanol (MEM.FCS) at 37°C with 10% CO₂ in air (pre-incubated spleen cells). The monolayers were washed twice in phosphate-buffered saline, overlaid with 2 ml of MEM.FCS and incubated for 5 min at room temperature before addition of responder cells (2×10^7) in 2 ml MEM.FCS. The dishes were centrifuged at 100g for 5 min in a prewarmed MSE Mistral 2L centrifuge and incubated for 1 h at 37°C with 10% CO₂ in air. The non-adherent responder cells were recovered from the dishes by brief agitation, washed once, and 2×10^7 resuspended in 9 ml MEM.FCS and transferred to a 25-cm² flask (Falcon no. 3013). Control cultures containing 2×10^7 unfractionated responder cells in 9 ml MEM.FCS were similarly prepared. To each flask 2×10^7 X-irradiated (1,000 R) B10 or B10.BR stimulator spleen cells were added in 1 ml MEM.FCS. All cultures were prepared in duplicate and incubated at 37°C for 5 d with 10% CO₂ in air. Cytotoxicity was determined using a microassay previously described¹⁸. Duplicate cultures were pooled, washed and resuspended in 0.6 ml RPMI 1640 supplemented with 10% v/v FCS. All T_c populations were titrated against ⁵¹Cr-labelled tumour target cells of both stimulator haplotypes. The results obtained were expressed as % lysis $\pm$ s.d. of triplicate determinations. Per cent specific lysis represents the % lysis minus the spontaneous release of label by target cells incubated with medium alone. Absorption of BALB/c (H-2^d) responders on: a, fresh B10 (H-2^b) monolayers; b, preincubated B10 monolayers; c, preincubated BALB/c monolayers. Comparison of the cytotoxicity generated against the homologous tumour target cell, when non-adherent responder cells were stimulated with B10 ($\circ \cdots \circ$), or B10.BR (H-2^k, $\square \cdots \square$) as an unrelated haplotype, with unfractionated controls similarly stimulated (B10 $\bullet \cdots \bullet$, B10.BR $\blacksquare \cdots \blacksquare$). To eliminate any effect of storage of the responder cell populations during the 4-h preincubation of monolayer cells, a separate, fresh responder population was prepared for those experiments in b and c; hence, the unabsorbed control values differ slightly in a from those in b and c.

Table 1 The ability of pre-incubated B10 and BALB/c monolayers specifically to deplete B10.BR T_c precursors reactive towards H-2^b or H-2^d alloantigens

Responder	Pre-incubated monolayer	Stimulator	Cytotoxic titre	
			H-2 ^b target	H-2 ^d target
B10.BR	—	B10	20.0	8.4
B10.BR	—	BALB/c	<1	55.7
B10.BR	B10	B10	<1	16.8
B10.BR	B10	BALB/c	<1	250.0
B10.BR	BALB/c	B10	50.1	5.0
B10.BR	BALB/c	BALB/c	<1	3.3

B10.BR responder cells were absorbed on monolayers of B10 or BALB/c preincubated spleen cells as described in Fig. 1. Non-adherent cells, or unfractionated responders as controls, were stimulated by either B10 or BALB/c alloantigen. The cytotoxic titre for a given T_c population was calculated as the reciprocal of the highest dilution of the T_c population which gave 15% specific lysis of the target concerned.

against H-2^d or H-2^b alloantigens by exposure to monolayers of preincubated BALB/c or B10 spleen cells, respectively. Similar results were obtained when CBA responder cells were used. The technique described thus seems applicable to several strain combinations.

Two further sets of experiments, by eliminating alternative explanations, strongly supported the suggestion that the results obtained are attributable to binding of T_c precursors to the absorbing monolayer. First, 10⁷ B10.BR cells which remained non-adherent after exposure to a monolayer of 4-h incubated B10 cells were added to an equal number of untreated, normal B10.BR spleen cells and cultured for 5 d with X-irradiated B10 stimulator cells. For comparison, a mixture of 10⁷ X-irradiated and 10⁷ normal B10.BR responder cells were cultured in the presence of X-irradiated B10 stimulator cells. Control cultures containing 10⁷ non-adherent and 10⁷ X-irradiated B10.BR responder cells were similarly stimulated. The results in Fig. 2 show that the non-adherent B10.BR cells did not significantly suppress ($P > 0.3$) the response of normal B10.BR spleen cells to stimulation by B10 alloantigens. These results argue against the reduction in T_c generation achieved by pre-exposure to an absorbing monolayer being attributable to induction of a suppressor population, or to blockage of the response by B10 cells or cell debris carried over from the monolayer.

Second, the reduction in T_c generation is not due to depletion of a proliferative, helper population which has been claimed to be necessary for T_c generation¹⁷. This was established by comparing ³H-thymidine incorporation and T_c generation of BALB/c cells which had been exposed to an absorbing monolayer of 4-h incubated B10 cells. The results in Table 2 show that, as expected, the non-adherent BALB/c cells were unable to mount a cytotoxic response when stimulated by B10 alloantigen but continued to show a normal T_c response to B10.BR alloantigen. However, the proliferation of the non-adherent BALB/c cells in response to B10 stimulation has not been impaired; thus, the B10 monolayer has not depleted this responding, helper-type population. This result has been confirmed in four other experiments. The controls in this experiment show that the non-adherent cells show no significant proliferation in the absence of any added stimulus, implying that significant carry-over of B10 antigen or of B10 cells capable of proliferation (or stimulation) has not occurred. This agrees with the immunofluorescence tests cited above which showed only a very low release of monolayer cells. Further corroboration that our results are attributable to specific binding of the T_c pre-

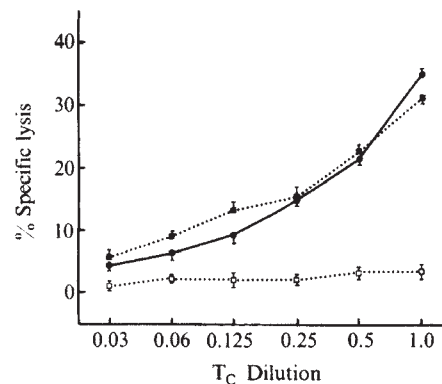


Fig. 2 Failure of B10.BR responder cells, non-adherent to pre-incubated B10 monolayers, to suppress the cytotoxic response of normal, unfractionated B10.BR responder cells to B10 alloantigens. 10⁷ B10.BR responder cells which remained non-adherent after exposure to a monolayer of 4-h incubated B10 cells, were added to an equal number of untreated, normal B10.BR spleen cells (■ · · · ■), and cultured for 5 d with X-irradiated B10 stimulator cells. Controls containing 10⁷ X-irradiated (1,000 R) and an equal number of normal B10.BR responder cells (● · · · ●), and 10⁷ non-adherent and 10⁷ X-irradiated B10.BR responder cells, (□ · · · □) were similarly stimulated. Cytotoxicity was determined by titration of the T_c populations against ⁵¹Cr-labelled H-2^b tumour target cells.

cursors by the absorbing monolayer comes from preliminary experiments in which T_c against the monolayer haplotype were generated by culturing the adherent cells.

In conclusion, the method outlined here of absorption on a monolayer of preincubated lymphocytes gives extensive haplotype-specific depletion of T_c precursors when subjected to the sensitive assay of *in vitro* induction of cytotoxic lymphocytes and has so far been successful in tests using various responder-stimulator combinations of eight mouse strains. The success of this method, in contrast to the variable results achieved in earlier reports, is due to the use of preincubated lymphocytes, which seem to form a more efficient absorbing monolayer. This could be due to a change in antigen presentation²⁴, which increases the binding affinity of T_c precursors, or to an increased binding of the adsorbent monolayer cells to the PLL-plastic, preventing dislodgement of any complex of bound T_c precursors and absorbing cell, or to a combination of both these factors.

Our results suggest two areas for further investigation. First, to define the change(s) induced by the pre-incubation of spleen cells that leads to effective T_c precursor binding, and second, to investigate whether GVH activity is affected by the removal of T_c precursors. However, the ability to deplete, and possibly recover, a population of T cells with restricted haplotype alloreactivity should be of immediate value in other areas of immunology, for example, in clarifying the role of major histocompatibility complex products in recognition by T cells²¹, or in evaluating the alternative models which have been proposed for alloreactive T-cell triggering²²⁻²⁴.

This work was supported by an Australian NH and MRC grant to W.B. H.Y.S. is supported by a Commonwealth Postgraduate Research Award. We thank Julie Ogilvie for technical assistance.

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Received 12 February; accepted 2 April 1979.

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Table 2 Absorption of BALB/c responder cells on pre-incubated B10 monolayers fails to reduce the proliferative response of the non-adherent responder cells towards B10 alloantigens

Responder	Preincubated monolayer	Stimulator	Proliferative response (³ H c.p.m.)	Cytotoxic titre H-2 ^b target	H-2 ^k target
BALB/c	—	B10	31,697 ± 2,786	16.7	2.0
BALB/c	—	B10.BR	43,967 ± 2,756	1.0	20.0
BALB/c	B10	B10	34,987 ± 2,625	<1	1.7
BALB/c	B10	B10.BR	49,457 ± 3,233	<1	21.3
BALB/c	B10	BALB/c	5,572 ± 416		
BALB/c	B10	Nil	5,147 ± 330		
BALB/c	—	BALB/c	4,905 ± 487		
BALB/c	—	Nil	4,709 ± 349		
Nil	—	BALB/c	90 ± 31		

BALB/c responder cells were absorbed on monolayers of pre-incubated B10 spleen cells as described in Fig. 1. 10⁷ Non-adherent cells, or 10⁷ unfractionated responders as controls, were stimulated by 10⁷ X-irradiated (1,000 R) B10, B10.BR or BALB/c stimulators, or none (nil), in 5 ml medium. To determine the proliferative response, duplicate 0.3-ml samples of each culture, after 3 d incubation, were transferred to a microtitre tray (Sterilin no. M29ARTL). Following centrifugation at 100g for 5 min, approximately half the supernatant was discarded and 25 µl of medium containing 0.5 µCi of ³H-thymidine was added to each well. After incubation in a humidified box for 18 h with 10% CO₂ in air, cultures were transferred to microtubes and collected according to the method of Smart *et al.*²⁰. Results obtained were expressed as ³H c.p.m., and represent the mean ± s.d. of quadruplicate determinations. Cytotoxicity was determined as described in Fig. 1 after 5 d incubation.

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Reciprocal expansions of idiotypic and anti-idiotypic clones following antigen stimulation

It has been postulated by Jerne that a network of idiotypic and anti-idiotypic interactions between lymphocytes may constitute a principal form of immune regulation^{1,2}. This network would control and 'remember' the antigen-induced activation of a particular (idiotypic) clone through its idiotypic or paratopic interactions with regulatory clones. These latter clones would in turn be regulated in the same fashion, creating what Jerne has called a 'web of V-domains' (ref. 2). Current experimental evidence strongly supports the concept of idiotype-directed regulation and also documents phenotypic complexity within the anti-idiotype response; for example, anti-idiotypic cells (and/or their products) may help or suppress the idiotypic response^{3–8}. However, this apparent complexity does not diminish the importance of Jerne's basic premise that reciprocal activation between idiotypic and anti-idiotypic clones could establish an equilibrium system capable of regulating the immune response^{9–11}. We present here evidence for oscillatory behaviour in the population dynamics of a single, antigen-stimulated clone and for the reciprocal behaviour of the specific anti-idiotypic (idiotype binding) clones.

Our studies used the T-15/phosphorylcholine system in BALB/c mice. These mice, immunised with a vaccine of strain R36a *Streptococcus pneumoniae* (Pn), produce an essentially monoclonal, T-independent antibody response. The idiotype (T-15) of this anti-Pn antibody is shared with the myeloma protein secreted by the BALB/c plasmacytoma, TEPC-15. Both are specific for the hapten phosphorylcholine (PC)¹². The kinetics and magnitude of the response of the T-15-bearing cells in the spleen was determined in two ways: (1) by the binding of radioactive [¹⁴C]PC and (2) by counting anti-Pn antibody plaque-forming cells (PFC) in agarose using R36a polysaccharide-coated sheep red blood cells (RBC) as indicator cells¹³. Both assays gave similar results; only the PFC numbers will be presented here. Serum antibody to Pn was measured by passive haemagglutination of antigen-coated sheep RBC. Changes in those clones recognising the T-15 idiotype (that is, the anti-idiotypic clones) were determined by the binding of the radiolabelled ¹²⁵I-myeloma protein, TEPC-15, on spleen cells. As a control, we used: (1) ¹²⁵I-MOPC-315 myeloma protein which has the same isotype (IgA) as TEPC-15 but which has both different idiotype and binding specificity; and, (2) ¹²⁵I-McPC-603, an IgA myeloma protein that binds PC but does not bear the T-15 idiotype¹⁴.

The time course of the idiotypic (T-15) and anti-idiotypic (T-15 binding) clonal responses after stimulation by Pn was visualised by the sequential immunisation of groups (3–4) of BALB/c mice (age- and sex-matched). Daily, for 14 d, a group of mice was immunised by an intraperitoneal injection of 20 µg of Pn vaccine. Thus, each group represented a unique stage of the anti-Pn response. On the 15th day, all immunised mice and a group of unimmunised mice were killed, and their spleens removed and disrupted into single cell suspensions. These cells

were washed three times in media and finally resuspended to a concentration of 10⁷ viable lymphocytes per ml. Duplicate aliquots (10⁶ cells in 0.1 ml) of these suspensions were then assayed as described above. This experimental schedule allowed mice at different stages of the anti-Pn response to be assayed simultaneously and using the same reagents. However, not more than three to four mice per interval could be handled. Therefore, to ensure the reproducibility of the data, we repeated the experiment four times, obtaining similar results in each trial.

As shown in Fig. 1, the accumulation of specific anti-Pn PFC in the spleen after a single injection of the antigen followed a cyclical pattern: a broad peak on day 5 (≈700 PFC per 10⁶ splenocytes) was followed by a second, sharper peak on day 12 (≈200 PFC per 10⁶ splenocytes). Specific binding of the hapten (PC) to the spleen cells coincided with the curve of the PFC, usually reaching a maximum 1 d before the PFC peak (data not shown). However, serum anti-Pn antibody titres did not reflect the cyclical pattern of splenic PFC; titres were highest around day 8 and then declined steadily.

The specific binding of ¹²⁵I-TEPC-15 by spleen cells from the immunised mice also underwent cyclical changes that seemed to be out of phase with the PFC curve (Fig. 1). Thus, the relative binding of T-15 (per cent of the control) rose to a maximum

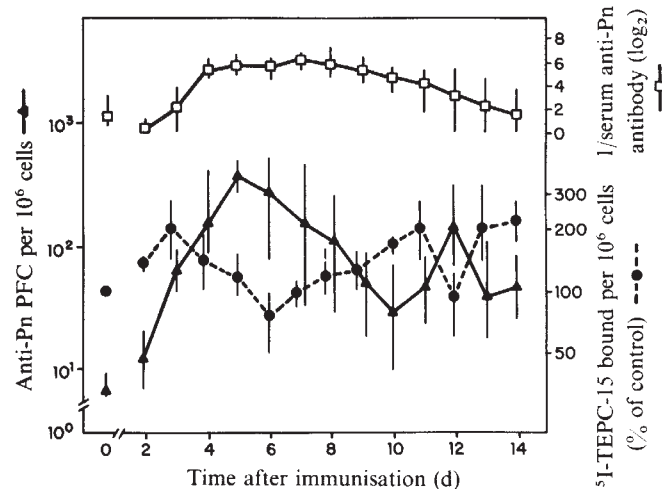


Fig. 1 Groups of three to five BALB/c mice were injected intraperitoneally with 20 µg of Pn vaccine at 2–14 d before removal of their spleens. Aliquots containing 10⁶ viable splenic lymphocytes were assayed for anti-Pn PFC (▲) and specific binding of ¹²⁵I-TEPC-15 myeloma protein (●). Serum, collected at the time of splenectomy, was assayed for anti-Pn antibody by passive haemagglutination of antigen-coated sheep RBC (□). Each symbol represents the mean of four experiments (12–15 mice). The vertical bars indicate the range of those experiments. For determination of ¹²⁵I-TEPC-15 binding, ascitic fluid from BALB/c mice bearing the plasmacytomas TEPC-15, MOPC-315 or McPC-603 was recovered and purified by (NH₄)₂SO₄ precipitation and gel (Sephadex G-200) filtration. Each purified protein was tested for its capacity to haemagglutinate Pn- or TNP-coated sheep RBC. Iodination was with radioiodinated Bolton-Hunter reagent (NEN), 5 mg protein per mCi ¹²⁵I. Iodinated proteins were equivalent to non-labelled proteins in their ability to haemagglutinate. To determine the optimal concentration of radioligand, spleen cells from BALB/c mice injected with TEPC-15 protein¹⁹ and then allowed to rest for 2 months were incubated with 10-fold dilutions of ¹²⁵I-TEPC-15 or ¹²⁵I-MOPC-315 0.1–100 µg protein. Saturation occurred at ≈1 µg for both proteins. Inhibition of binding (68–75%) occurred in the presence of 100 µg cold homologous ligand but not with the heterologous ligand or bovine serum albumin. To measure T-15 binding, duplicate tubes containing 10⁶ cells in 0.1 ml of ice-cold Hank's balanced salt solution containing 2% FCS were incubated at 4 °C for 10–12 h with ≈1 µg (0.05 ml) of labelled TEPC-15, MOPC-315 or McPC-603. The cells were then washed three times in cold Hank's containing 10% FCS and the radioactivity of the cell pellet was determined as c.p.m. Variation within duplicates was routinely less than 15%. Specific TEPC-15 binding is defined as: (c.p.m. bound ¹²⁵I-TEPC-15) – (c.p.m. bound ¹²⁵I-MOPC-315 or ¹²⁵I-McPC-603). In any single experiment, specific TEPC-15 binding was computed by the subtraction of only one of the control proteins. Because the c.p.m. of bound MOPC-315 or McPC-603 remains constant (see text) throughout the 14-d experimental period, both serve equally well as specificity controls. The specific TEPC-15 binding is then expressed as per cent of binding by spleen cells from the control group of unimmunised mice (control = 100%).

Table 1 ^{125}I -TEPC-15 binding in the presence of excess McPC-603*

	0	3	4	6	8	9	10	12
T-15 + McPC-603	100	111	125	86	164	147	127	79
Average T-15 bound	100	190 (120-260)	150 (120-180)	74 (50-100)	110 (90-160)	115 (75-145)	160 (150-195)	90 (60-125)

* Groups containing three age- and sex-matched BALB/c mice were immunised as described in the text. On day 0 mice were killed and from each group a suspension of pooled spleen cells was made in ice-cold RPMI-1640 media with HEPES buffer (pH 7.3). The cells were washed three times in fresh media and resuspended to a concentration of 10^7 viable lymphocytes per ml. Aliquots of 0.1 ml of the appropriate cell suspension were then added to duplicate tubes containing 3 μg of ^{125}I -TEPC-15 and 100 μg of McPC-603. Cells were incubated overnight at 4 °C and then washed three times in Hank's balanced salt solution containing 10% fetal calf serum (FCS) (pH 7.2). The recovered cell pellets were then counted as described. Binding is expressed as per cent of the day 0 binding value. T-15 binding was measured as described in the text. Binding is expressed as per cent of the day 0 control and the range of four experiments is indicated in parentheses.

(two- to threefold) on day 3, declined steadily until day 6, and then increased to a second maximum by days 10-11, which immediately preceded the second PFC peak (day 12). T-15 binding fell on day 12, then increased again to the third maximum value on days 13-14.

The probe for T-15 binding cells—the radioiodinated TEPC-15 myeloma protein—could bind in at least three ways: by the Fc fragment, by the PC-specific binding site and by the T-15 idiotope. Thus, the relative changes in T-15 binding on splenocytes following Pn immunisation could result from members of Fc receptor-bearing cells or from residual antigen. The MOPC-315 and the McPC-603 myeloma proteins acted as controls for these variables; in particular, the McPC-603 protein has the PC binding paratope and IgA isotype but lacks the T-15 idiotope¹⁴. The binding of the control probes, MOPC-315 and McPC-603, remained relatively constant ($\pm 5\%$ of the day 0 value) over the 14-d period following immunisation with Pn. The biphasic pattern of T-15 binding was retained even when cells were incubated with ^{125}I -TEPC-15 in a large excess of McPC-603 protein (Table 1). Thus, it is likely that the T-15 probe predominantly measured anti-idiotypic cells.

Our data on PFC oscillation agree with previous results showing a cyclical pattern of *Escherichia coli* lipopolysaccharide-specific PFC in mice¹⁵ and PFC against human immunoglobulin in rabbits¹⁶. In the latter study, PFC peaks were observed on days 6 and 12 after immunisation and, as in our experiments, this oscillation was not reflected in the serum antibody levels.

Earlier work from Nisonoff's laboratory has demonstrated the sequential activation of antigen-reactive clones during the immune response¹⁷. To ensure that the T-15 idiotype dominated both anti-Pn PFC peaks, we carried out a plaque-inhibition assay using a rabbit anti-T-15 antiserum (rendered specific by absorptions with: (1) pooled murine IgM; (2) MOPC-315; and (3) C57BL/6 spleen cells). The addition of this serum at a dilution of 1:500 inhibited both the day 6 and day 12 BALB/c anti-Pn PFC response by $\geq 80\%$. The C57BL/6 anti-Pn response was inhibited by $\leq 9\%$ and the BALB/c anti-TNP response was unaffected.

The increase of T-15 binding by splenic lymphocytes (day 3) suggests an expansion of the anti-idiotypic clones, presumably through cell proliferation. Nonetheless, alternative explanations for the increased binding, such as shedding and passive uptake of T-15 receptors or a trapping of circulating, receptor-bearing lymphocytes in the spleen, cannot be excluded *a priori*. The expansion of the anti-idiotypic clones seems to be cyclical. However, the decrease in T-15 binding observed between the peaks may be partly due to occupation of the receptors by anti-Pn antibody. Because the serum anti-Pn titres do not oscillate, it is more likely that this would result from antibody production *in situ*. Within the micro-environment of the spleen, local concentrations of idiotype antibody might be sufficient to occupy a significant number of receptors on idiotype-binding cells. That the second peak of T-15 binding (day 10) does reflect an expansion of the anti-idiotypic clone rather than a mere unmasking of the receptors for the idiotype is supported by Cosenza's finding of anti-T-15 PFC during the immune response to Pn¹⁸. These PFC appeared on days 7-8 after immunisation,

following the decline of anti-Pn PFC. We find that at this time the T-15 binding begins to increase and continues to do so until days 10-11; presumably, the binding assay detects cells other than PFC.

Our data demonstrate the periodic expansion of an antigen-activated idiotype clone associated with a reciprocal expansion and diminution of cell-associated, anti-idiotypic (idiotype binding) activity. The pattern is consistent with the hypothesis of a regulatory network based on an idiotype-anti-idiotypic equilibrium. It is tempting to speculate that the out-of-phase expansions of antibody-forming cells and cell-associated anti-idiotypic activity result from the interaction of the idiotype clone with its anti-idiotypic regulators.

We thank Dr J. Novotny for discussions and Ms C. Berman for technical assistance and acknowledge the gift of strain R36a *S. pneumoniae* by Dr H. Kohler. G.K. is the recipient of a Rockefeller Foundation Fellowship. The research was supported in part by American Cancer Society grant IM-35 and by USPHS grant CA 14922 from the NCI.

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Received 12 January; accepted 2 April 1979.

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A possible mechanism for insulin resistance and hyperglycaemia in NZO mice

THE New Zealand obese (NZO) mouse is characterised by obesity, hyperinsulinaemia, insulin resistance and mild glucose intolerance¹⁻⁸. It has been thought of as a model of the adult-type human diabetes mellitus, but neither the genetics of the NZO syndrome nor its basic cause have been elucidated. The metabolic disturbances found in these mice, for example

Table 1 Effect of NZO serum on insulin binding to normal mouse liver membranes

	Control serum	NZO serum				
		1:2	1:4	1:8	1:16	1:32
¹²⁵ I-insulin specifically bound (% of total)	21.5	23.9	8.5	11.6	13.1	17.0

Approximately 100 µg of mouse liver membrane (10-week-old NIH white mice) was exposed to 50-µl dilutions of NZO serum in pooled control serum for 1 h at 24 °C. The membranes were then pelleted in a Beckman Microfuge and washed twice by resuspension in 0.1 M sodium phosphate buffer at 4 °C. ¹²⁵I-insulin (50 pg; ~13,000 c.p.m.) in 100 µl of 0.1% bovine albumin-0.1 M sodium phosphate buffer, pH 7.4, was added to the washed membranes, with or without excess unlabelled insulin (10 µg) and the mixtures incubated for 90 min at 15 °C. Membranes were pelleted, washed and counted for ¹²⁵I-insulin radioactivity in a gamma counter. Radioactivity bound in the presence of excess unlabelled insulin (nonspecific binding) was subtracted to give specific binding. Results are the means of two experiments each performed in triplicate.

increased rates of lipogenesis⁹⁻¹² and defects in insulin secretion^{8,13,14}, could well be secondary to chronic hyperinsulinaemia and hyperglycaemia. Because the NZO strain appeared to share a common ancestry with the NZB model of systemic autoimmune disease^{1,2,15} we felt that the metabolic syndrome in the NZO mouse might have an immune basis. It was originally suggested that the insulin resistance of NZO mice might be due to an antagonist to the action of insulin². We now report that NZO serum contains a globulin, probably an autoantibody, which inhibits insulin binding and indirectly immunoprecipitates the solubilised insulin receptor.

Initial studies were performed with pooled NZO sera from mice of both sexes older than 12 months. We then developed our own inbred colony from mice derived from the original stock of Bielschowsky and Bielschowsky¹. The mice had free access to food and water and became obviously obese by 8-10 weeks of age. Orbital sinus blood samples were obtained between 9 and 10 a.m. from 5-20-week-old animals in the post-absorptive state.

The effect of NZO serum on insulin binding was studied by preincubating a source of insulin receptors (NZO, BALB/c or NIH mouse liver membranes, human placental membranes, or cultured IM-9 lymphocytes) with NZO serum serially diluted in control mouse serum (NZW, BALB/c, NIH or CBA mice), followed by washing and measurement of specific ¹²⁵I-insulin binding. The preparation of receptors^{16,17}, and biologically active ¹²⁵I-insulin¹⁸, and the binding technique¹⁹, have been described previously. Preincubation of normal mouse liver

Table 2 Insulin binding to normal mouse liver membranes preincubated with serum from two NZO mice, before and after treatment of each serum with anti-mouse κ antiserum

	¹²⁵ I-insulin specifically bound (% of total)	
	Untreated	Treated
Control serum	12.3	12.1
NZO-3	2.5	6.0
NZO-4	5.9	10.3

Serum (75 µl) from male NZO mouse no. 3, female NZO mouse no. 4 and a normal NIH mouse pool was incubated with 75 µl of rabbit anti-mouse κ antiserum (Miles, 64-366 lot 11) or with 75 µl of non-immune rabbit serum for 18 h at 4 °C. Immune complexes in the samples treated with anti-κ serum formed visible precipitates after centrifugation in a Beckman Microfuge. The supernatants were aspirated, diluted a further 1:2 in normal mouse serum (final dilution 1:4) and then tested for an inhibitory effect on ¹²⁵I-insulin binding, according to the protocol described in Table 1. Results are the means of two experiments each performed in triplicate.

membranes with dilutions of NZO serum resulted in inhibition of subsequent ¹²⁵I-insulin binding (Table 1). For unknown reasons, undiluted serum either had no effect or actually increased binding slightly. Inhibition was specific for ¹²⁵I-insulin and was not seen for ¹²⁵I-growth hormone. However, preliminary experiments have shown a smaller effect of NZO serum on binding to NZO membranes, which may be due to antigenic modulation by previous exposure to antibody as the affinity of the receptor in NZO membranes is impaired (data not shown). Maximum inhibition of binding was 20-80% of control and was seen at dilutions between 1:4 and 1:32. Inhibition was never observed at serum dilutions greater than 1:64. Re-exposure of membranes to fresh serum did not cause a further decrease in binding (data not shown). Similar results were obtained in heterologous systems using either human placental membranes or cultured human IM-9 lymphocytes as receptor sources. Treatment of NZO sera with anti-mouse κ antiserum partially removed the inhibitory activity (Table 2), suggesting that the inhibitory factor was an immunoglobulin.

An immunoprecipitation assay for receptor antibodies was performed by incubating serum with Triton-solubilised mouse liver or human placental membranes pre-labelled with ¹²⁵I-insulin, followed by addition of a second (precipitating) antibody^{20,21}. Solubilised mouse liver receptors were immunoprecipitated by NZO serum in a dose-dependent manner, after addition of anti-mouse globulin antiserum (Table 3). Precipitation of ¹²⁵I-insulin radioactivity in excess of control was not observed with NZO sera in the absence of receptor, thereby excluding the possibility of antibodies to insulin itself.

Table 3 Immunoprecipitation of ¹²⁵I-insulin-labelled receptors by NZO serum and anti-mouse globulin antiserum

	Control serum	NZO serum			
		1:10 ³	1:10 ²	1:20	1:10
c.p.m. (% of total)	1.2	1.2	1.7	3.5	5.9

Normal mouse liver membranes (~50 mg ml⁻¹) were solubilised by mixing them with 1% Triton (v/v)-0.1 M sodium phosphate buffer, pH 7.4, for 1 h at 24 °C. The mixture was then centrifuged at 200,000g for 1 h. 20 µl (~80 µg of protein) of the clear supernatant containing solubilised insulin receptors was incubated for 1 h at 24 °C with a trace amount of ¹²⁵I-insulin (50 pg; ~13,000 c.p.m.) in a total volume of 180 µl of 0.1 M sodium phosphate buffer. Pooled NZO serum (20 µl) serially diluted in control NZW serum, or control serum alone, was then added and incubations continued for a further hour at 24 °C. The mixtures were cooled to 4 °C and 50 µl of rabbit anti-mouse globulins (Behring Diagnostics lot A2929C) was added for a further 4 h at 4 °C. Immune complexes, which formed a visible suspension, were precipitated by centrifugation in a Beckman microfuge, washed once with 0.1% Triton buffer and counted in a gamma counter. When the receptor was omitted from the initial Triton buffer-¹²⁵I-insulin mixture, radioactivity subsequently present in the immune precipitate was equivalent to that in the control, thus excluding the presence of antibodies to ¹²⁵I-insulin itself. Results are the means of two experiments each performed in triplicate.

Immunoprecipitation was more sensitive than the binding inhibition assay, activity being detected up to serum dilutions of 1:100 with titres (serum dilutions causing half-maximum immunoprecipitation) ranging from 1:10 to 1:40. We attempted to determine the class of antibody involved by using a variety of second antisera to mouse immunoglobulins A, G and M. Antiserum to mouse IgA was ineffective. Weak immunoprecipitation was obtained in the majority of sera tested by using either anti-mouse 7S globulins or anti-mouse IgM. We are investigating the possibility that the receptor antibody in NZO sera may not be well recognised by commercially available antisera, or may be a low molecular weight IgM.

A number of lines of evidence favour an immune basis for the metabolic abnormalities in NZO mice. First, the mice are distantly related to the autoimmune NZB strain^{1,2,15}. Second, the Bielschowskys² demonstrated improvement of obesity, hyper-

glycaemia and insulin resistance during pregnancy and after treatment with stilboestrol. Although pregnancy is a state of 'physiological' insulin resistance it is also a state of 'immunological inertia'²², which might explain its beneficial effect in an autoimmune disease. Remission after stilboestrol therapy may have been due to the immunosuppressive effect of the steroid, particularly as gonadectomy was without effect in either sex². Finally, the finding in NZO mice of a relatively high incidence of spontaneous and carcinogen-induced tumours²³ would be consistent with an underlying immune disorder.

The relationship of the antibody to age, sex and metabolic status is now being studied to define the pathological role of the antibody and to determine whether the NZO model is the counterpart of the recently described human syndrome of insulin-resistant diabetes associated with antibodies to the insulin receptor^{19,21}.

We thank Dr Richard Larkins for NZO mice sera, Dr Lieselotte Herberg for mice derived from the Bielschowsky stock, Dr Jesse Roth for support and advice and Mrs Beverly Knight for secretarial assistance. This work was initiated while L.C.H. was a recipient of a C. J. Martin Fellowship from the National Health and Medical Research Council of Australia. A.I. was supported by a grant from the Juvenile Diabetes Foundation.

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Received 22 November 1978; accepted 3 April 1979.

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FSH induction of functional LH receptors in granulosa cells cultured in a chemically defined medium

THE rat ovarian granulosa cell is an excellent model for studying the mechanisms and control of hormone-dependent cell differentiation. During graafian follicle development, the granulosa cells sequentially develop specific membrane receptor sites for follicle-stimulating hormone (FSH)^{1,2} and luteinising hormone (LH)³. *In vivo* studies on the mechanism of granulosa cell differentiation have established that FSH induces the

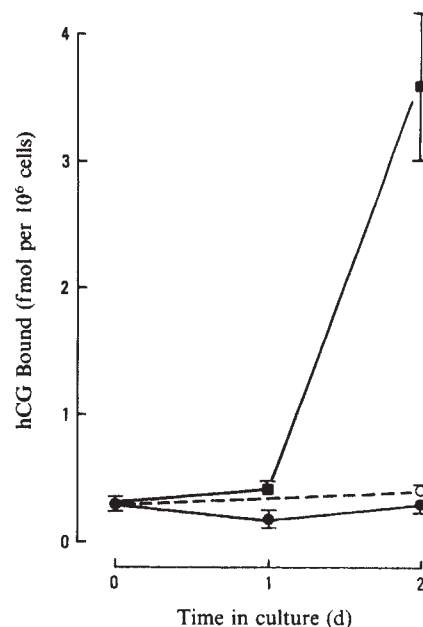


Fig. 1 FSH induction of hCG binding sites in rat granulosa cells cultured in a chemically defined medium. ●, Without FSH (in the presence or absence of serum); ○, with FSH and 10% hypophysectomised female rat serum; ■, with FSH in the absence of serum. Immature female rats (Sprague-Dawley, 23–25 d old) were hypophysectomised and implanted with a 10-mm silastic capsule containing diethylstilboestrol (DES) to stimulate granulosa cell proliferation. Five days after hypophysectomy, the ovaries were removed and the granulosa cells collected and cultured as previously described⁹. The culture medium consisted of McCoy's 5a medium (modified; GIBCO) supplemented with 2 mM L-glutamine, 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin sulphate. The following agents were added where indicated; purified oFSH (Papkoff G4-150C; FSH potency = 50 × NIH-FSH-SI U per mg; LH potency < 0.01 NIH-LH-SI U per mg); androstenedione (10⁻⁷ M; substrate for aromatisation) and 10% animal serum from immature hypophysectomised female rats. The granulosa cells were cultured for 2 days in a 95% air–5% CO₂ incubator (37 °C). After the incubation, the granulosa cells were scraped from the dish with a rubber policeman, washed twice with 5-ml portions of Dulbecco's phosphate buffer with 0.1% bovine serum albumin (BSA) and the final pellet resuspended in the same buffer. Cell number was determined using a haemocytometer. Highly purified hCG (CR-119; 11,600 IU per mg) was iodinated by the procedure of Greenwood *et al.*¹⁰. The specific activity (80,000–130,000 c.p.m. per ng) and maximal binding capacity (40–45%), were determined by radioligand receptor assay using rat testicular membranes¹¹. Samples (50 µl) of granulosa cells (1 × 10⁶ cells) were incubated for 16 h (23 °C) with 100 µl of ¹²⁵I-hCG (~1 × 10⁻¹⁰ M) with and without unlabelled hCG (100 IU Pregnyl)¹². The absence of androstenedione from media did not modify the FSH effect, indicating that FSH alone induced the LH/hCG receptors. Data points indicate mean ± s.e. of six separate experiments with five dishes per determination per experiment.

appearance of the LH receptor sites in the granulosa cell^{4,5}. The FSH-induced increase in LH receptors is essential for preparing the graafian follicle for the pre-ovulatory surge of LH which initiates ovulation and subsequent luteinisation of the granulosa cells. Studies of cultured granulosa cells have led to the suggestion that the FSH induction of LH receptors is not a direct process but requires an interaction between the granulosa cell and other ovarian cell types, a concept consistent with the known importance of cell–cell interaction in cell differentiation⁶. This hypothesis stemmed from the observation that LH receptors can be induced by FSH in granulosa cells *in vivo* and in organ cultures of intact ovarian follicles, but not in isolated granulosa cells cultured as monolayers in medium containing serum^{7,8}. We show here that the inability of previous workers^{7,8} to induce LH receptors in isolated granulosa cells may have been due to the use of serum in their tissue culture medium. We demonstrate that specific, high-affinity LH/hCG receptors can be induced by FSH in isolated granulosa cells cultured in a chemically defined medium, but not in isolated granulosa cells cultured with serum. In addition, we show that these receptors are capable of mediating important steroidogenic responses.

As shown in Fig. 1, the hCG-binding capacity of freshly collected granulosa cells was very low (0.32 ± 0.014 fmol per 10^6 cells; 192 ± 8 sites per cell; mean $\pm$ s.e. of six separate experiments). Culturing granulosa cells for 2 days in medium containing purified ovine FSH and 10% serum from hypophysectomised female rats (or serum from horse, pig or fetal calf), did not increase the ^{125}I -hCG-binding capacity as compared with untreated control cultures or freshly isolated granulosa cells (Fig. 1). In contrast, culturing granulosa cells in serum-free medium containing FSH resulted in a dramatic increase in ^{125}I -hCG binding as compared with control cells (Fig. 1). A small increase in binding was observed at day 1, after which the binding capacity increased 10-fold, to reach 3.61 ± 0.60 fmol per 10^6 granulosa cells ($2,095 \pm 325$ sites per cell) by day 2.

Figure 2a shows that the hCG receptors induced by FSH are hormone specific. Increasing concentrations of unlabelled hCG, but not prolactin, competed with ^{125}I -hCG for binding sites in a dose-dependent manner. Moreover, unlabelled FSH inhibited binding only at doses 1,000 times higher than those of hCG, an inhibition that can be explained by contamination of the FSH preparation with LH. Scatchard analysis of the binding data (Fig. 2b) suggested the presence of two classes of ^{125}I -hCG-binding sites. This is consistent with our previous studies using granulosa cells from adult rats¹². The calculated K_d (1.4×10^{-11} M) of the high-affinity binding site (Fig. 2b) is of the same order as that determined in previous studies with rat granulosa cells^{3,12}. Furthermore, FSH treatment for 2 days *in vivo* increased ^{125}I -hCG binding in the granulosa cell to a level comparable with that found in the present *in vitro* study (unpublished result).

To determine if the LH/hCG receptors induced by FSH were functionally active, we investigated the effects of purified LH and FSH on the biosynthesis of progesterone and oestrogen (Table 1). In this experiment, the granulosa cells were cultured for 2 days in a chemically defined medium with and without LH or FSH, washed, and then recultured for another 2 days in the presence and absence of the purified gonadotropins. During the 0–2 and 2–4 day culture period, FSH caused a marked stimulation of oestrogen and progesterone production, whereas LH had little effect, indicating that the granulosa cells are responsive to FSH, but not to LH. However, after 2 days of FSH priming LH caused a highly significant ($P > 0.001$) increase in both oestrogen and progesterone production compared with the control (FSH; control) cultures. This result shows that the LH/hCG receptors induced by FSH are functionally active. The present finding that purified LH stimulates oestrogen formation is important as it demonstrates for the first time that LH, like FSH, can stimulate aromatase enzyme activity in the granulosa cell.

Table 1 Induction by FSH of the ability of purified LH to stimulate progesterone and oestrogen production by isolated rat granulosa cells *in vitro*

Treatment 0–2 d; 2–4 d	Progesterone (ng ml ⁻¹)		Oestrogen (ng ml ⁻¹)	
	Day 0–2	Day 2–4	Day 0–2	Day 2–4
Control; Control	0.10 $\pm$ 0.01*	0.22 $\pm$ 0.01	< 0.04	< 0.04
LH; LH	0.50 $\pm$ 0.01	0.27 $\pm$ 0.01	0.19 $\pm$ 0.01	0.07 $\pm$ 0.01
FSH; FSH	21.67 $\pm$ 1.92	8.35 $\pm$ 0.71	4.28 $\pm$ 0.22	4.09 $\pm$ 0.22
FSH; LH	—†	7.32 $\pm$ 0.47‡	—	2.49 $\pm$ 0.14‡
FSH; Control	—	1.73 $\pm$ 0.13	—	1.11 $\pm$ 0.10

Granulosa cells (1×10^5 viable cells) were cultured for 4 days in McCoy's 5A medium with 10^{-7} M androstenedione, either alone or with purified Papkoff ovine FSH (100 ng ml^{-1}) or purified oLH (100 ng ml^{-1} ; Papkoff; G3-222B; LH potency = $2.75 \text{ NIH-LH-SI U per mg}$; FSH potency = $0.001 \text{ NIH-FSH-SI U per mg}$). After 2 days the media were collected, and the granulosa cells were washed twice with 2-ml portions of McCoy's 5A medium. The cells were then recultured for 2 days in 1 ml of fresh medium containing 10^{-7} M androstenedione and hormones where indicated. Progesterone and oestrogen in the media were measured by radioimmunoassay¹³.

* Mean $\pm$ s.e.; $n = 8$.

† Same values designated in the FSH (day 0–2).

‡ Significantly different from control group (FSH; control) ($P < 0.001$) as determined by analysis of variance.

The present results demonstrate that purified FSH can induce the appearance of specific, high-affinity LH/hCG receptors in isolated granulosa cells and that the LH/hCG receptors are capable of mediating two important biological responses, namely, oestrogen and progesterone biosynthesis. This direct action of FSH in the granulosa cell can explain the increased responsiveness of the developing pre-ovulatory follicle to LH during the follicular phase of the reproductive cycle. The fact that the characteristics of the LH/hCG receptors (K_d and number of binding sites) induced in the granulosa cell by FSH *in vitro* are similar to those induced by FSH *in vivo*, suggested that the present *in vitro* observations reflect a normal physiological event. Our finding that FSH induces the appearance of LH/hCG receptors in isolated granulosa cells cultured in a chemically defined medium (without serum) strongly suggests that this basic action of FSH does not require the participation of other ovarian cells, as recently proposed by Nimrod *et al.*⁷.

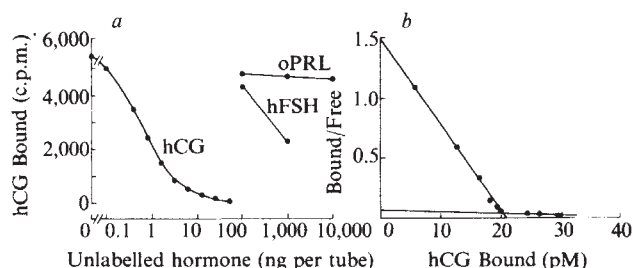


Fig. 2 Specificity and binding characteristics of LH/hCG receptors induced by FSH *in vitro*. *a*, Specificity of ^{125}I -hCG binding to granulosa cells after 2 days of culture with oFSH (100 ng ml^{-1}) in chemically defined medium (without serum). Granulosa cells (1×10^6 cells) (prepared as described in legend to Fig. 1) were incubated in $300 \mu\text{l}$ (phosphate buffered saline–0.1% BSA) buffer for 16 h (23°C) with $20,000 \text{ c.p.m.}$ of ^{125}I -hCG and the indicated amounts of unlabelled hCG (CR-119), purified human FSH (NIH-FSH-HSI) or ovine prolactin (NIH-P-S12). Data points represent the mean of four determinations. *b*, Scatchard analysis of binding data from displacement curve shown in *a*. K_d , Dissociation constant = 1.4×10^{-11} M; number of binding sites = 3,157 sites per cell.

Our failure to induce LH/hCG receptors with FSH in granulosa cells cultured in medium containing serum is consistent with previously published data^{7,8,14}. Moreover, our results are consistent with those of an earlier study¹⁴ which reported an FSH stimulation of LH binding in cultured porcine granulosa cells, although the data from these cells were variable and complicated by the loss of pre-existing LH receptors during the incubation. The nature of the inhibitory effect of serum is unknown; however, it may result from the presence of factors in serum which have been reported to inhibit FSH binding to receptors¹⁵.

Experiments with animal cells in culture have usually used serum because the maintenance and growth of functionally differentiated cell cultures require certain factors found in serum¹⁶. However, the addition of serum to culture media has made it difficult, sometimes impossible, to interpret the action of specific regulatory substances. We conclude that the *in vitro* culture system described here offers an important model for investigating and defining the control mechanisms of hormone-dependent differentiation of a normal mammalian cell in a chemically defined medium.

This work was supported by NCI DHEW grant 1-R01-CA-21867-01, Rockefeller Foundation grants RF-76001 and RF-75029, the WHO and NIH research program project grant HD-12303. We thank Professor S. S. C. Yen for his interest, Professor H. Papkoff for providing the purified oFSH and oLH, Dr R. E. Canfield and the Center for Population Research of the NICHD for providing the purified hCG, the Distribution Officer of NIAMD for the NIH-FSH-HSI and ovine prolactin NIH-P-S12, Ms Sally Ng, Linda Tucker and Cheryl Fabrics for

technical assistance and Ms Cheri Anderson for typing the manuscript.

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Glucocorticoids inhibit expression of Fc receptors on the human granulocytic cell line HL-60

GLUCOCORTICOIDS decrease levels of circulating peripheral lymphocytes and immunoglobulins, inhibit mitogen- and antigen-induced blastogenesis of cultured lymphocytes and decrease bactericidal activity of granulocytes and other phagocytic cells¹⁻⁴. Fc receptors on granulocytes are thought to play a vital part in adherence of antibody-coated particles to the surfaces of these cells, a step which initiates phagocytosis⁵. Factors which regulate the expression of the Fc receptor could therefore influence phagocytic capability, and it has been suggested that glucocorticoids might act in such a way^{6,7}. We report here that glucocorticoids inhibit expression of the Fc receptor on the human promyelocytic cell line HL-60 (ref. 8). This inhibition is not accompanied by increased cell death, reduced proliferation or a general reduction in protein synthesis.

For assay of Fc receptors, IgG1 was purified from the serum of a patient with multiple myeloma. The precipitate obtained by 45% saturation with ammonium sulphate was dissolved in acetic acid-ethylene diamine buffer (0.1M, pH 7.0) and desalted by passing through a column (1×20 cm) of Biogel P-100 (Bio-Rad). The excluded peak from the column was placed on a column (1×15 cm) of Sephadex QAE-50 (Pharmacia) and eluted with acetic acid-ethylene diamine buffer. The IgG1 obtained was pure by immunoelectrophoresis, and contained only traces of other proteins by gradient gel and cellulose acetate electrophoresis. The protein was iodinated with ¹²⁵I (Amersham, 11-17 Ci μg⁻¹) by the solid phase lactoperoxidase method^{9,10}. Protein concentration was determined by absorbance at 280 nm ($A_{280}^{1\%} = 11.4$). Specific activities were between 20 and 57 Ci mmol⁻¹.

This labelled IgG1 was used to determine the number and affinity of Fc binding sites by the modification of the competitive binding assay of Unkeless and Eisen¹¹ described in the legends to Figs 1 and 2. The assay showed high specificity for the Fc fragment of IgG1. In competition experiments with 0.5-5 nM

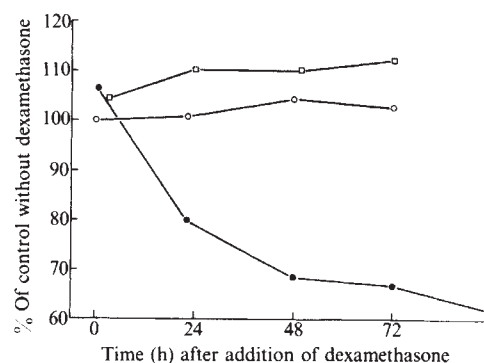


Fig. 1 Time course with HL-60 cells of the effect of 200 nM dexamethasone on Fc receptor sites per cell (●), leucine incorporation (□) and viable cell counts (○). HL-60 cells⁸ were cultured in RPMI 1640 (GIBCO) with 10% fetal calf serum, penicillin (50 units ml⁻¹) and gentamycin (50 μg ml⁻¹) at 37 °C in humidified room air containing 5% CO₂. Dexamethasone dissolved in medium at 0.1 mM was added at time zero to three flasks containing 50 ml cell suspension (2×10⁵ cells per ml). An equivalent amount of medium was added to three control flasks. At 15 min and 24, 48 and 72 h, 4×10⁶ cells were removed and washed twice with 15 ml Dulbecco's phosphate-buffered saline (GIBCO) containing 1 mg ml⁻¹ bovine serum albumin (2× recrystallised, Calbiochem) (PBS-BSA) at room temperature. The cells were incubated for 30 min in 15 ml PBS-BSA (2-8×10⁵ cells per ml) at 37 °C with 60 c.p.m. shaking to dissociate endogenous immunoglobulins in fetal calf serum from the Fc receptors. They were then resuspended in PBS-BSA at 5×10⁷ cells per ml, and 20 μl aliquots were incubated in duplicate with 20 μl of ¹²⁵I-labelled IgG1 (40 nM), with and without unlabelled IgG1 (1 μM) in albuminised 1.5-ml conical polypropylene Eppendorf centrifuge tubes (Brinkman) for 30 min at 37 °C with 60 c.p.m. shaking. At the end of the incubation the cell suspension was pipetted over 350 μl fetal calf serum at 0 °C in 400-μl microfuge tubes (Brinkman) and centrifuged for 30 s at 10,000g. The supernatant was rapidly aspirated and the tips of the tubes containing the cell pellet counted in a gamma counter at an efficiency of 70% for ¹²⁵I. The difference between the c.p.m. bound in the presence (an almost negligible amount, as shown in Fig. 2) and absence of 1 μM competing unlabelled IgG1 (that is, the c.p.m. corresponding to saturably bound IgG1) was calculated as molecules per cell. Since at 40 nM IgG1 nearly saturates the Fc receptor sites (see Fig. 2) this value gives a close estimate of the total number of Fc receptor sites per cell. Leucine incorporation was measured by removing 100-μl aliquots from each of the flasks and incubating for 4 h at 37 °C under 5% CO₂ in humidified room air in microtitre plates (Costar) with 100 μl of a solution (3 μCi ml⁻¹) of ³H-leucine (NEN, 297 Ci mol⁻¹) in GIBCO minimum essential medium. Cells were collected on glass fibre filters using a multiple automated sample harvester, and after drying were counted in a toluene-based scintillation fluid at an efficiency of 50% for ³H. Viable cell counts were determined as the product of the fraction viable by Trypan blue exclusion and the cell count determined with a Coulter counter. Viabilities of control and dexamethasone-treated cells were not significantly different, and always remained above 95%. Each point represents the mean for determinations on three control flasks and three flasks with dexamethasone. For the controls, the number of Fc receptor sites remained essentially constant at about 16,000 sites per cell over the whole time period; the rates of leucine incorporation increased slightly, and the viable cell counts increased from 200 to 650 cells per μl.

¹²⁵I-labelled IgG1 the Fc fragment, prepared by papain hydrolysis and affinity chromatography, at 10 nM reduced binding of labelled IgG1 by 50% and at 100 nM by 90%, as effectively as unlabelled IgG1. The Fab fragment, similarly prepared, did not compete at all. Human IgG3 competed as well as IgG1, whereas IgG2 and IgG4 competed weakly and IgA did not compete. Protein A (Pharmacia) at 100 nM reduced binding by 50%. It failed to reduce binding further at concentrations as high as 50 μM, suggesting among other possibilities that there may be more than one class of Fc receptor on HL-60 cells.

Figure 1 illustrates the time course of effects of 200 nM dexamethasone on HL-60 cells. Within 24 h there was a significant decrease in the number of Fc receptor sites, which by 48-72 h had fallen to ~65% of the controls without steroid. In other experiments the levels reached varied from 75 to 40%. They remained low for at least 8 d in the continued presence of dexamethasone, but returned to normal within 48 h of removal of the steroid. Over the 72-h period there was no decrease in overall leucine incorporation, so there was no general reduction of protein synthesis. Similarly, cell proliferation was not decreased.

The reduction in the number of Fc receptor sites was not associated with a change in the affinity of the receptor for IgG1. Control cells and cells treated with 1 μ M dexamethasone for 72 h bound IgG1 with the same dissociation constant of 10 nM (Fig. 2).

Prednisolone and cortisol at 1 μ M after 48 h reduced Fc receptor sites from 14,400 to about 8,000 per cell. Oestradiol and progesterone at 1 μ M had no effect. Dexamethasone gave a half-maximal response at 10 nM, comparable to effects of dexamethasone on other cellular processes¹². This concentration is consistent with a process mediated by binding to glucocorticoid receptors in these cells, since it corresponds with the dissociation constant we have determined separately for binding of dexamethasone to glucocorticoid receptors in HL-60 cells at 37 °C. These cells contain numbers of glucocorticoid receptor sites similar to myeloblasts from patients with acute myelogenous leukaemia¹³. After Fc receptors are reduced to about 50% by treatment with trypsin, their reappearance is almost completely blocked by glucocorticoids. In the absence of steroid they return to normal after 48 h.

In two previous studies^{4,14}, effects of glucocorticoids on Fc receptors in human monocytes were investigated. The only effect detected, a decrease in IgG binding, required steroid concentrations of 0.1–1 mM, far above pharmacological concentrations, and the effect was not specific for glucocorticoids¹⁴. The duration of exposure to steroids, 90–120 min, was much less than we find necessary to produce significant effects. Furthermore, the immune adherence techniques used would probably not detect effects of the kind we describe. Methods involving rosette formation with antibody-coated red cells or binding of fluorescence-labelled immune complexes can detect cells with more than some undetermined threshold level of Fc receptors (probably 10,000–20,000 sites per cell), but do not permit accurate quantitation of binding.

Our finding that glucocorticoids reduce the number of Fc receptors on HL-60 cells is important in at least two ways. First, it establishes in a pure cell line an effect that is manifested on a discrete membrane structure and that, in contrast to what is often observed¹², is not accompanied by general inhibition of protein synthesis. Second, it may provide a molecular explanation for the suppressive effects of glucocorticoids on the phagocytosis and destruction of autologous tissue that characterise

some immune responses mediated by Fc receptors. The observation^{16,17} that patients with diseases such as autoimmune haemolytic anaemia and autoimmune thrombocytopenia frequently show clear improvement and decreased phagocytic activity in their granulocytes after treatment for 24–48 h with glucocorticoids could well be explained by a decrease in the number of Fc receptors. The time course is too rapid to be accounted for by known effects on human immunoglobulin levels² but is similar to that needed for the reduction in Fc receptors that we describe here. The assay for Fc receptors may also prove useful for studying other factors which regulate this immunologically important structure.

We thank Drs Robert Gallo, Robert Gallagher and Steven Collins for providing HL-60 cells. These studies were supported in part by grants CA 17323, AM 03535 and CA 17643 from the NIH and by the Core Grant (CA 23108) of the Norris Cotton Cancer Center.

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Received 16 October 1978; accepted 4 April 1979.

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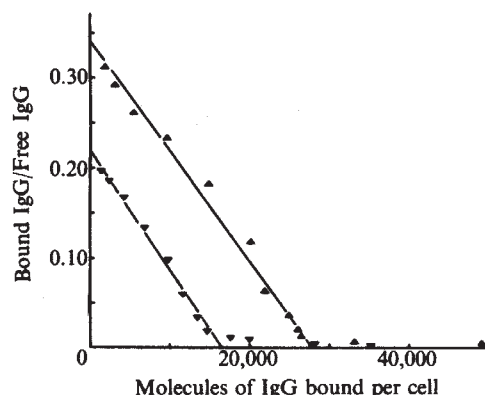


Fig. 2 Scatchard plot of binding of IgG1 to Fc receptors of HL-60 cells after incubation with 1 μ M dexamethasone. ∇ , Controls without dexamethasone. Δ , Cells incubated with 1 μ M dexamethasone. Cells were incubated with or without dexamethasone for 96 h and washed in PBS-BSA as described for Fig. 1. They were then resuspended at 1.6×10^6 cells per ml in PBS-BSA. Aliquots were incubated as described for Fig. 1 with 125 I-labelled IgG1 at 12 concentrations from 100 pM to 1 μ M. After the incubation the tubes were centrifuged at 10,000g for 10 s and 20 μ l supernatant removed and counted to determine the final concentration of free labelled IgG1. The tubes were then cooled to 3°C, 1.2 ml of PBS-BSA at 0°C was added and the cells were resuspended. After 5 min the tubes were centrifuged at 12,000g for 4 min, the supernatant aspirated and the tips counted as described for Fig. 1 to determine bound 125 I-labelled IgG1. The data are plotted by the method of Scatchard¹⁵. Nonsaturably bound IgG1 was not subtracted for these results. Bound IgG and free IgG on the ordinate are given in units of c.p.m. per 20 μ l of cell suspension; the values on the ordinate therefore represent the bound IgG1 as a fraction of the free IgG1 at equilibrium.

Negative and positive inotropic action of vanadate on atrial and ventricular myocardium

VANADATE (vanadium in the +5 oxidation state) occurs widely in animal tissues¹, and has been suggested to be the first regulatory agent of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$; it inhibits ATPase preparations from kidney and red blood cells at very low concentrations^{2–4}. Hackbarth *et al.* have shown that vanadate produces positive inotropic effects in isolated cat papillary muscles⁵, raising the question of whether this positive inotropic effect is accompanied by an inhibition of myocardial $(\text{Na}^+ + \text{K}^+)\text{ATPase}$, and, if so, whether both effects occur at similar concentrations of vanadate. We report here that we have observed a striking difference in the inotropic actions of vanadate on isolated heart preparations. Although the force of contraction was reduced in atria, it was increased in ventricular myocardium. This dissociation correlated well with changes in transmembrane potential but not with inhibition of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ isolated from both atrial and ventricular myocardium.

Left and right atria and papillary muscles from guinea pig and cat, and atrial and ventricular trabeculae of the cow, were mounted in glass chambers for measuring isometric contraction. The bathing solution (20 ml), containing (mmol l⁻¹) NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.6, NaHCO₃ 24.9, NaH₂PO₄ 1.2

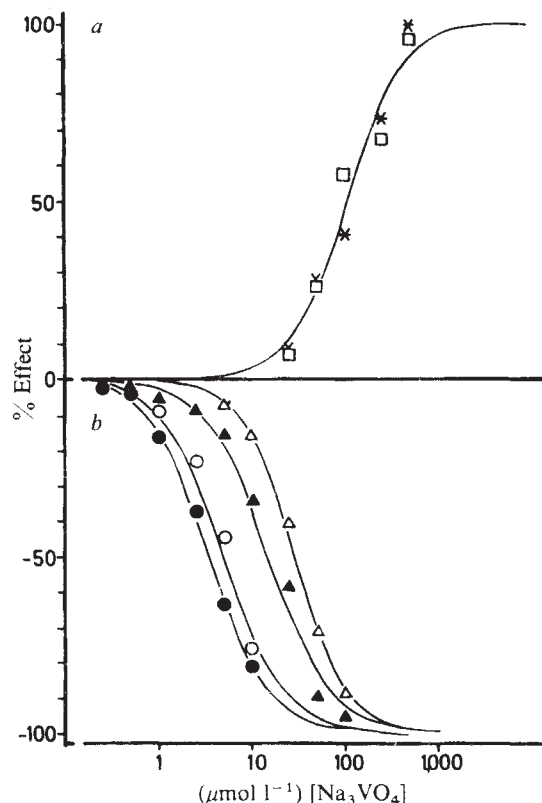


Fig. 1 Change in the force of contraction in papillary muscle (a) and atria (b). ED_{50} values ($\mu\text{mol l}^{-1} \pm \text{s.e.m.}$) were calculated by fitting the logit function to the experimental data using the least-square method: papillary muscle, guinea pig ($\square$) 110.3 ± 1.9 , cat (*) 124.1 ± 2.6 ; left atria, guinea pig ($\circ$) 4.73 ± 0.10 , cat (Δ) 28.1 ± 0.47 ; right atria, guinea pig ($\bullet$) 3.29 ± 0.06 , cat ($\blacktriangle$) 14.8 ± 0.28 . The curves were plotted according to the equation

$$y(x) = \frac{100}{1 + 10^{-s(x - \log ED_{50})}}$$

where s = slope and x = log concentration⁶.

and glucose 11.1, was equilibrated with 95% O_2 and 5% CO_2 (pH 7.4) at 31 °C. Increasing doses of Na_3VO_4 (Fluka) were added to the bathing solution cumulatively every 15 min. The preparations were driven electrically by platinum electrode stimulation at 1 Hz (guinea pig) or 0.5 Hz (cat, cow). Pulse duration was 1 ms (intensity 20% above threshold, preload of atria 10^{-2} N, that of papillary muscles and trabeculae 7×10^{-3} N mm^{-2}). Action potentials of the isometrically contracting atrial and ventricular tissues were recorded with glass micro-electrodes filled with 3 mol l^{-1} KCl (resistance 10–30 M Ω) using a horizontal Perspex chamber, perfused with increasing concentrations of Na_3VO_4 .

Figure 1 shows the inotropic action of vanadate. In a concentration range of 25–500 $\mu\text{mol l}^{-1}$, vanadate increased the force of contraction of papillary muscles. In contrast, it strongly decreased the force of contraction of stimulated left atria and spontaneously beating right atria. The effect appeared immediately on perfusion and steady-state conditions were reached within 10 min. On washing with drug-free bathing solution, the force of contraction of the atria increased above control levels and regained initial values after about 15 min. At high concentrations of vanadate ($>1 \text{ mmol l}^{-1}$) and 1 Hz stimulation frequency, a small increase in basal tension of guinea pig papillary muscles was observed, but without arrhythmic activity.

Figure 2 shows the corresponding action potentials. In guinea pig papillary muscles 500 $\mu\text{mol l}^{-1}$ vanadate broadened the action potential by about 15% at both 30 and 90% repolarisation, whereas in left atria 50 $\mu\text{mol l}^{-1}$ vanadate produced a shortening of the action potential by 60 and 70% at 30 and 90%

Table 1 Enzyme activities of bovine and guinea pig heart preparations and the inhibitory effect of orthovanadate on the Na^+ , K^+ -ATPase

Beef	Activity	Mg^{2+} -ATPase ($\text{Na}^+ + \text{K}^+$)ATPase $\mu\text{M Na}_3\text{VO}_4$	Atria	Ventricle
			5.4 ± 1.3 12.7 ± 2.3 0.59 ± 0.14	7.9 ± 2.4 21.5 ± 3.2 0.60 ± 0.14
Guinea pig	Activity	Mg^{2+} -ATPase ($\text{Na}^+ + \text{K}^+$)ATPase $\mu\text{M Na}_3\text{VO}_4$		
			48.1 ± 12.8 6.9 ± 2.4 0.75^*	30.9 ± 10.4 23.9 ± 2.6 0.62 ± 0.13

Membrane ATPase preparations were derived using the method of Akera *et al.*⁷. Enzyme activities were determined with a coupled spectrophotometric assay⁸, in the presence of 50–100 μg enzyme protein and (mmol l^{-1}) Na^+ 100, K^+ 10, Mg^{2+} 5 and ATP 3, at 37 °C, pH 7.4, and were measured in $\mu\text{mol PO}_4$ per mg protein per h. The Mg^{2+} -ATPase activity was measured in the presence of 1 mmol l^{-1} ouabain. Each activity is the result of four separate preparations determined in triplicate and each ID_{50} value the result of four determinations (with exception*, where $n = 1$) prepared from 48 guinea pigs. Values are means $\pm$ s.e.m.

repolarisation, respectively. The upstroke velocity of the action potential of both atria and papillary muscles remained nearly unchanged. Similar results were obtained from atrial and ventricular preparations of cat and bovine heart. The time course of the vanadate effect on transmembrane potentials correlated with that of the change in inotropy.

The specific activities of the Mg^{2+} - or $\text{Na}^+ + \text{K}^+$ -activated ATPase for bovine atrial and ventricular tissues are shown in Table 1. Despite the difference in enzyme activities, concentration–response curves for the action of sodium orthovanadate gave similar ID_{50} values for the inhibition of bovine atrial and ventricular $\text{Na}^+ + \text{K}^+$ -activated ATPase. Guinea pig ventricular and atrial ($\text{Na}^+ + \text{K}^+$)ATPase was inhibited to a similar extent,

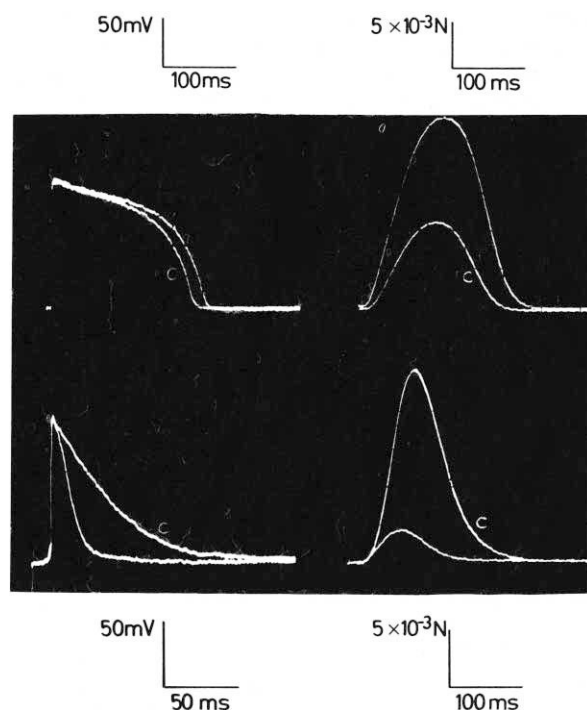


Fig. 2 Action potentials from guinea pig papillary muscle (left upper trace) and from the left atrium (left lower trace) in control conditions (C) and under Na_3VO_4 (14 min 500 $\mu\text{mol l}^{-1}$ for papillary muscle and 8 min 50 $\mu\text{mol l}^{-1}$ for the atrium). The related force of contraction in control and test conditions is shown in the right traces. The upstroke velocities of the action potentials were not significantly influenced by vanadate, being 150 Vs^{-1} for the papillary muscle and 197 Vs^{-1} for the atrium.

although the atrial enzyme activity was too low to allow more than one estimation in a preparation using 96 atria.

The positive and negative inotropic actions of vanadate are accompanied by a similar inhibition of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ of ventricular and atrial preparations, despite a higher activity of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ from ventricular myocardium. Negative inotropic changes occur at concentrations ($1\text{--}100\ \mu\text{mol l}^{-1}$) which induce a 55–95% inhibition of bovine cardiac $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ preparations and a 15–60% inhibition of rubidium uptake in red blood cells⁴, whereas positive inotropic changes ($50\text{--}500\ \mu\text{mol l}^{-1}$) correspond to a 90–100% inhibition of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ preparations and a 55–80% inhibition of rubidium uptake⁴. It remains to be explained why high concentrations of vanadate ($>1\ \text{mmol l}^{-1}$), which strongly inhibit $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ preparations, do not produce arrhythmias similar to those observed with high doses of cardiac glycosides.

Positive and negative inotropic effects correlate well with a broadening and shortening of the action potential, respectively. Therefore, we conclude that differing inotropic effects of vanadate are mainly due to the alteration of the transmembrane potential, but not primarily to the inhibition of cardiac $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. Another striking property of vanadate is its concentration-dependent stimulatory effect on the adenylate cyclase of rat fat cell membranes⁵. It is still possible that the positive inotropic action of vanadate on ventricular myocardium is caused by an increase in intracellular cyclic AMP. However, the negative inotropic effect of vanadate in atria which occurs at lower concentrations seems to be due to a shortening of the action potential.

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Received 27 February; accepted 9 April 1979.

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Endogenous digitalis-like activity in the plasma of the toad *Bufo marinus*

CARDIAC GLYCOSIDES derived from plants have a major role in the pharmacotherapy of cardiac disease, and, because they bind to and inhibit $(\text{Na}^+ + \text{K}^+)\text{ATPase}$, are important in studies of ion transport mechanisms. In the animal kingdom, structurally related compounds with cardiotonic activity have been shown to exist in the poison glands of bufonid toads¹, but this area has received little attention. Recently we developed a sensitive radioreceptor assay for the detection of endogenous 'digitalis-like' agonists in animals². This assay depends on the ability of a substance to compete with ^3H -ouabain for binding to $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ on human red cells. Using this assay, together with assays for inhibition of K transport and $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ activity, we found high concentrations of 'digitalis-like' activity in the skin of several species of Amphibia². Although this activity is presumably present in skin as a protective toxin, it is possible that it might, under some circumstances,

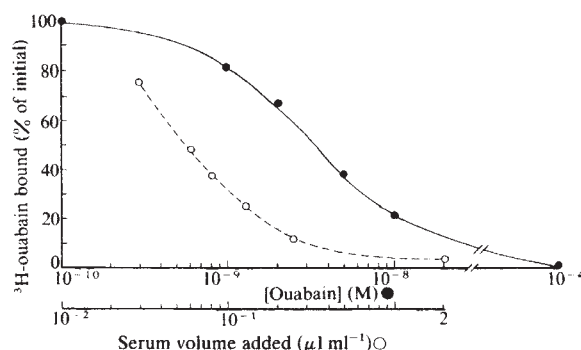


Fig. 1 Effect of ouabain (●) or serum (○) on the binding of ^3H -ouabain to human RBC was assessed by a modification of the method of Gardner and Conlon⁴. Heparinised blood was washed 4 times with 140 mM choline chloride at 4°C, the buffy coat was removed and RBC were resuspended at a haematocrit of 4% in an assay buffer of 140 mM NaCl, 30 mM HEPES and 10 mM glucose, pH 7.4. To 900 μl of this RBC suspension was added 100 μl of various dilutions of ouabain or serum and these mixtures were incubated at 37°C for 1 h. Cells were then sedimented at 1,500 r.p.m., followed by resuspension in 225 μl of the fresh assay buffer containing $5 \times 10^{-10}\ \text{M}$ ^3H -ouabain ($8\ \text{Ci mmol}^{-1}$, NEN). After incubation at 37°C for 70 min, bound and free ouabain were separated by washing 4 times in microfuge tubes with 140 mM choline chloride, followed by addition of 10% perchloric acid to the pellet and inversion of the tube into a scintillation vial containing Aquafluor (NEN). Each assay contained duplicate tubes in which ^3H -ouabain binding was carried out in the presence of $10^{-4}\ \text{M}$ unlabelled ouabain. This was taken to represent nonspecific binding, and was always less than 10% of total ^3H -ouabain bound. The per cent of ^3H -ouabain specifically bound to each group of cells was determined by subtracting the per cent bound to cells in the presence of $10^{-4}\ \text{M}$ ouabain from the per cent actually bound in each experimental tube. This specific binding for each tube was divided by the specific binding to cells which were never exposed to serum or unlabelled ouabain, and this value was multiplied by 100, to give the value plotted on the vertical axis. The points which are plotted represent the mean of duplicates which varied less than 5%. The ouabain concentration on the abscissa represents the concentration in 1.0 ml of preincubation mix.

also function as an endogenous inhibitor of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. We now demonstrate, using both receptor and immunoassays, that high concentrations of digitalis-like activity are present in the plasma of the toad, *Bufo marinus*. As cellular $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ would presumably be exposed to this endogenous digitalis-like activity, it may have an important, but as yet undetermined, function in this species.

Pre-exposure of human red blood cells (RBC) to dilutions of *Bufo marinus* serum followed by washing and resuspension in fresh buffer, caused a dose-dependent inhibition of subsequent ^3H -ouabain binding to these cells. The dilution curve produced by serum was parallel to that produced by unlabelled ouabain (Fig. 1), 50% inhibition of ^3H -ouabain binding being caused by a 1/20,000 dilution of serum and 95% inhibition being caused by a 1/1,000 dilution. Assuming that the activity has a binding affinity for human RBC $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ that is similar to that of ouabain, the apparent serum concentration in five toads ranges from 2 to $5 \times 10^{-5}\ \text{M}$. *Rana pipiens*, whose skin has little or no detectable activity (J. S. F. and Daly, in preparation), also had no serum activity detectable, even when tested at 1/10 dilution (data not shown). The digitalis-like activity was unaffected by heating to 100°C for 15 min, but 95% of the activity was lost after dialysis for 48 h.

To characterise the ouabain binding sites on the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ of bufonid RBC, we washed cells six times in 15 volumes of buffer to remove circulating digitalis-like activity, and then performed a ^3H -ouabain binding assay as with human RBC. No specific ^3H -ouabain binding was detectable over a range of tracer concentrations from 5×10^{-10} to $5 \times 10^{-8}\ \text{M}$, whereas binding was easily detectable in the same number of human RBC (data not shown).

Serum from *Bufo marinus* cross-reacted strongly in a digitalis immunoassay. Serial dilutions produced displacement nearly parallel to that produced by digitalis itself (Fig. 2). In contrast, human serum produced no displacement. The data indicate that 1 μ l of bufonid serum contains 0.05 ng of immunoreactive digitalis-like activity.

Preincubation of bufonid serum with antibodies to either digitalis or ouabain blocks the receptor competition (Fig. 3). Thus the 3 H-ouabain binding-inhibitory activity seems to be caused by the same molecular species that cross-reacts with antibodies to digitalis and ouabain. This immunological evidence represents entirely independent confirmation that bufonid serum contains high concentrations of a digitalis-like activity.

These data suggest that the concentration of serum digitalis-like immunoactivity is $\sim 10^{-7}$ M. When compared to the concentration as estimated by competition for receptor binding ($2-5 \times 10^{-5}$ M), it seems that the receptor binding activity of this molecule has been better conserved than has its cross reactivity with anti-digitalis and anti-ouabain antibodies. Direct structural studies will determine its precise relationship to the cardiac glycosides, and to known bufotoxins.

Several questions remain unanswered by these studies. First, although digitalis-like activity is present by both receptor-competition and immunological criteria, it remains to be shown that this serum activity is capable of inhibiting the activity of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. Studies suggest that the serum activity is a fully potent inhibitor of the human enzyme (J. S. F. unpublished results). Second, it is not certain by what means *Bufo marinus* can survive with a serum concentration equivalent to more than 1,000 times the concentration of digitalis that produces toxicity

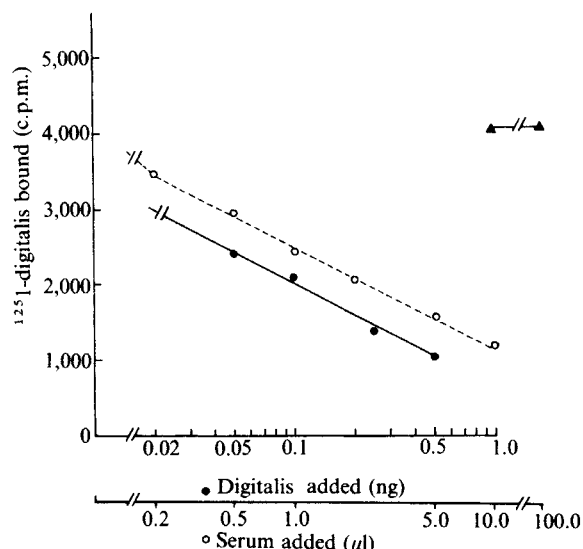


Fig. 2 Dilution curves for digitalis (●) and bufonid serum (○) in a radioimmunoassay for digitalis. The immunoassay was carried out using a kit supplied by Corning Medical. Reagents include rabbit anti-digoxin antibody which is covalently linked to glass particles and suspended in phosphate-buffered saline with 1% bovine serum albumin, and ^{125}I -digoxin tracer, comprising ^{125}I -tyrosine linked to the digoxin molecule. The assay is carried out at room temperature in a total volume of 500 μ l. The indicated amounts of digitalis are added to the assay tubes in 100 μ l of pooled human serum to develop the dilution curve for digitalis. The indicated volumes of bufonid or human serum (▲) are similarly added to the assay tubes, with the difference between the indicated volume and 100 μ l being added as 140 mM NaCl. After incubation for 30 min at room temperature, glass beads were centrifuged at 1,600g for 10 min, supernatants decanted, and radioactivity in the pellets counted in a gamma counter. Points represent the mean of duplicates which varied less than 5%. Very similar results were obtained with serum from two other *Bufo marinus* toads. In this assay, digitoxin shows 3% cross-reactivity, and ouabain, testosterone, progesterone and spironolactone show negligible cross-reactivity.

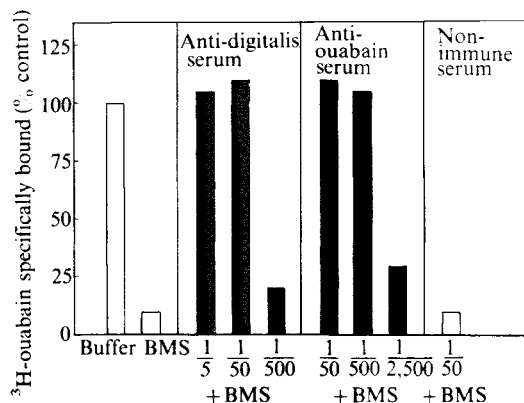


Fig. 3 Effect of anti-digitalis and anti-ouabain antisera on the ability of bufonid serum to inhibit ^3H -ouabain binding to human RBC. *Bufo marinus* serum (BMS) was diluted 1/1,000 in an assay buffer of 140 mM NaCl, 30 mM HEPES and 10 mM glucose, pH 7.4. A 200 μ l aliquot of this BMS was incubated with 50 μ l of buffer, or with 50 μ l of sheep anti-digitalis serum, rabbit anti-ouabain serum or normal human serum which had been diluted in assay buffer to yield the final dilution indicated on the figure. A control tube comprised assay buffer alone. These tubes were incubated at 37°C for 1 h, followed by the addition of 750 μ l of human RBC which had been washed 4 times in 140 mM choline chloride and then suspended in assay buffer, to give a final haematocrit of 5%. After an additional incubation for 1 h at 37°C, cells were sedimented at 1,500 r.p.m., followed by resuspension in 225 μ l of fresh assay buffer containing 5×10^{-10} M ^3H -ouabain (8 Ci mmol $^{-1}$, NEN). After a final incubation at 37°C for 70 min, bound and free ouabain were separated by washing 4 times in microfuge tubes with 140 mM choline chloride, followed by addition of 10% perchloric acid to the pellet and inversion of the tube into a scintillation vial containing Aquaflo NEN. Each assay contained duplicate tubes in which ^3H -ouabain binding was carried out in the presence of 10^{-4} M unlabelled ouabain. This was taken to represent nonspecific binding, and was always less than 5% of total ^3H -ouabain bound. The data shown are representative of two other experiments not described.

in man. *Bufo marinus* is known to be resistant to the effects of cardiac glycosides, requiring 100 times more ouabain to stop its heart *in vivo*, or to inhibit Na^+ transport across its bladder *in vitro* than does *Rana pipiens*⁴. This relative resistance to ouabain is consistent with our observation that tracer ^3H -ouabain binds poorly to bufonid cells. Recent experiments suggest that the bufonid enzyme binds ouabain with low affinity (J. S. F., unpublished results).

Although *Bufo* is clearly relatively resistant to the action of digitalis, high concentrations of this substance, in the range which we estimate (by receptor assay) to be present in its serum, inhibit short circuit current across the bladder of this species *in vitro*³. For this reason, it is possible that sufficient activity is bound to the enzyme at prevailing concentrations to tonically inhibit ATPase activity. Preliminary studies demonstrate that bladder from *Bufo marinus* contains easily detectable digitalis-like activity by receptor assay and immunoassay. Studies are in progress to determine what fraction of this extractable activity is actually bound to the bufonid $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. This should be of interest because the toad bladder is often used as a model system for Na^+ transport in the distal nephron of the mammalian kidney. Perhaps it accounts in part for the observation that short circuit current across the toad bladder *in vitro* increases during equilibration of the bladder in buffer.

Thus we have demonstrated for the first time that a digitalis-like substance circulates naturally in the plasma of an animal. The effect of this circulating digitalis-like activity on the physiology of *Bufo marinus* is currently unclear, and the possibility that it tonically inhibits or regulates the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ in this species needs further investigation.

We thank Betsy Marsh for technical assistance and Dr Vincent Butler for antibodies to digitalis and ouabain. Toads were obtained from Connecticut Valley Biologic Supplies.

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Received 27 November 1978; accepted 15 March 1979.

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Circular hydrogen bonds

CIRCULAR hydrogen bonds present a new, experimentally demonstrated principle showing how hydration water molecules and hydroxyl groups of macromolecules can cooperate to form a network-like pattern. Quantum chemical calculations show that chain-like H-bonds in the crystal lattice¹ are energetically favoured above individual ones^{2,3}. This is due to the cooperative effect, which leads to increased H-bonding activity of an OH-group if it is already accepting or donating an H-bond. These linear structures can close up to form circular arrangements comprising four and more OH-groups^{4–6}. Such circles have actually been described for crystal structures of ice⁷ and of ice clathrates⁸, but in these cases the water molecules within the circles are related by crystallographic symmetry elements and are therefore not independent of each other. One should expect to find lattice-independent circular H-bonds in crystal structures of large O—H-rich molecules which co-crystallise with water of hydration, conditions which are satisfied by the cyclodextrin family. The smallest member, α -cyclodextrin (α -CD; cyclohexaamylose), consists of six α (1,4)-linked glucose molecules and contains six primary and 12 secondary hydroxyl groups ((C₆H₁₀O₅)₆, molecular weight 973). From aqueous solution, α -CD crystallises as hexahydrate, α -CD·6H₂O, and this complex, with a total of 120 hydroxyl groups (4 × 18 from α -CD and 4 × 12 from the six H₂O) in one unit cell has been studied by X-ray and neutron diffraction methods (ref. 9, and Klar, Hingerty and W.S., unpublished). Two other complexes, a second modification of the hexahydrate (K. Lindner and W.S., unpublished) and α -CD·methanol·4H₂O (ref. 10) have been investigated by X rays. As refinement in all cases is around $R = 4\%$ for data extending to 0.89 Å resolution, hydrogen atom positions could be assigned. I discuss here results obtained from the X-ray/neutron study of α -CD·6H₂O.

Figure 1 shows a small, relevant section of the crystal structure of α -CD·6H₂O obtained from the X-ray/neutron study. Three circular H-bonded structures can be distinguished, one six-membered and two five-membered, all connected by water molecules W(1) and W(4). Two chain-type, linear H-bond structures extend on the left and right hand sides, linked with the circles by W(2) and W(4). Both chain structures with asymmetrical lengths of seven and eight O—H groups are formed by unidirectional O—H···O bonds which indicate the cooperative effect. As similar chains occur in small as well as in large molecule crystal structures¹, they can be considered as general structural elements.

The three H-bonded circles shown in Fig. 1 all differ from each other. The six-membered circle I is formed by three interconnected water molecules W(1), W(2), W(4) and by three α -CD hydroxyl groups of which O(2)2 and O(3)3 belong to the same molecule and O(2)3 is from a symmetry-related α -CD. The six oxygen atoms form a distorted hexagon in an approximate boat shape, with W(1) and O(3)3 in bow/stern positions. In

this circle, H-bond cooperation is again effective because all O—H···O bonds run in the same direction. Therefore, this kind of circle is called *homodromic*.

The adjacent five-membered circles II and III comprise water molecules W(1) and W(4). Circle II contains three hydroxyl groups, of which two, O(2)6 and O(3)1, originate from one α -CD molecule, and O(6)2 is from a symmetry-related molecule. W(4) acts as a double H-bond donor and thus generates two ···O—H···O—H chains which collide at the double acceptor O(3)1. Because this circle consists of two counter-running chains, it is called *antidromic* (circles with more randomly orientated chains would be called *heterodromic*). In circle III, in addition to water molecules W(1) and W(4), a third water, W(3), is enclosed and the two hydroxyl groups belong to two different α -CD molecules. This circle is also *antidromic*, as W(3) is a double donor and W(1) a double acceptor for H-bonds. The H···O(6)6 distance, 2.72 Å is unusually long because this hydrogen is also bonded to O(6)1B at 2.85 Å and thus forms a bifurcated H-bond. Based on the sum of the van der Waals potential minimum radii, 1.50 Å for H and 1.65 Å for O, all H···O distances < 3.15 Å can be called bonding interactions and the H···O(6)6, O(6)1B distances are well within this range¹¹.

It is remarkable that the three circles share the two water molecules W(1), W(4). The latter are not only used to fill empty gaps between the α -CD molecules but they are also fully integrated into the H-bonding scheme. We can assume that the circular H-bonds are made possible through the water molecules which, because of their quadruple functionality and their low pK value ($pK = 7$) relative to the α -CD hydroxyl groups ($pK \sim 12.3$ (refs 12,13)), can act as connectors and as shunts. The commonly observed tetrahedral arrangement of the H-bonded ligands is barely satisfied. Only W(2) and W(4) show approximate tetrahedral configuration (with O···W···O angles in the range 94° to 139°); the tetrahedron around W(1) is very distorted (angular range 66° to 142°) and the ligands around W(3) are in a planar-trigonal arrangement.

Quantum chemical studies on the three circles described here have shown that the total energy in each circle is about 2–4 kcal per circle smaller than the sum of the corresponding individual H-bonds (Lesyng and W.S., unpublished). The gain in energy on formation of circular H-bonds is thus similar to that calculated

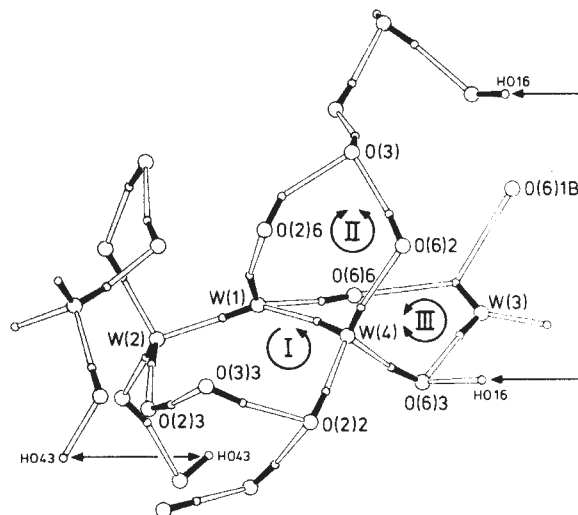


Fig. 1 A section of the α -CD·6H₂O crystal structure showing circular and chain-like H-bonds. The latter are marked by arrows, the former by roman numerals I–III. Circular arrows indicate the direction of the O—H···O hydrogen bonds. Circle I is homodromic and circles II, III are antidromic. Water and α -CD hydroxyl oxygens are indicated by W and O, respectively; for further numbering see ref. 10. Ranges for O···O; H···O distances (Å) and O—H···O angles in H-bond circle I are 2.692–3.016 Å; 1.75–1.95 Å; 169–176°; in circle II, 2.747–3.021 Å; 1.86–2.14 Å; 150–171°, in circle III, 2.831–3.339 Å; 1.71–2.72 Å; 117–171°.

for chain-like H-bonds^{2,3}, but the latter are more frequently encountered because the former are obviously restricted to large unit cells containing many hydroxyl groups and water molecules. Circular H-bonds might exist at the surfaces of large, hydrophilic molecules where, in combination with isolated and with chain-like H-bonds, they can link existing OH-groups or other hydrophilic residues using water molecules as connectors and shunts. It is likely that a pattern of circular and linear H-bonds can form, providing the basis for more extensive hydration shells. It is also tempting to regard the flickering cluster structure of bulk water¹⁴ and the clathrate shells around hydrophobic molecules⁸ as a population of predominantly five- and six-membered circular H-bonds.

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Received 14 December 1978; accepted 2 April 1979.

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Calcium-dependent regulation of protein synthesis and degradation in muscle

MUSCLE proteins undergo continuous intracellular turnover as do proteins in other cells^{1,2}. Furthermore, hormones, nutrients and work load can alter rates of protein synthesis and degradation in muscle, resulting in growth or atrophy of these tissues^{1,2}. In hereditary muscular dystrophies, where there is prominent wasting of the affected tissues, rates of both total protein synthesis and degradation are elevated^{3–10}. The immediate cause of this muscle atrophy is the imbalance resulting from an increase in protein degradation which exceeds a smaller enhancement in average protein synthesis. No simple explanation based on a defect in the response of dystrophic cells to known hormonal or nutritional factors has satisfactorily explained the elevation in the rates of both protein synthesis and degradation. We have now investigated the possible role of increased cellular Ca^{2+} as a mediator of such changes in protein metabolism based on other known structural and biochemical alterations in dystrophic muscles^{11,12}. Lesion(s) involving membranes in muscle as well as other cells occur in hereditary dystrophies, including the main human form, Duchenne dystrophy^{11,12}. One characteristic of the dystrophic plasma membrane seems to be an increased permeability to the high concentrations of Ca^{2+} normally present in extracellular fluid^{11,12}. In addition, studies have suggested a decreased ability of sarcoplasmic reticulum to sequester Ca^{2+} in dystrophic muscles^{13,14}. Thus, it is possible that increased Ca^{2+} might be responsible for the stimulation of both protein synthesis and degradation which occurs in these muscles. To test this idea, we have experimentally increased the uptake of external Ca^{2+} into rat muscles by using the divalent cation ionophore, A23187.

The ability of this ionophore to increase the transport of Ca^{2+} across membranes has resulted in its application as a widely used tool for the study of many Ca^{2+} -dependent cellular processes¹⁵. The experiments reported here demonstrate that increased movement of Ca^{2+} into muscle can produce effects which closely resemble dystrophic muscle and that the increased net catabolism can be reversed by certain factors.

We have studied the effect of A23187 in paired, contralateral rat soleus muscles incubated *in vitro* in defined media. Previous studies have shown that such isolated muscle preparations remain viable and maintain linear rates of both protein synthesis and degradation for at least several hours^{16,17}. In these conditions, when tension is not maintained, rates of degradation exceed synthesis. For measurements of protein synthesis, incorporation of ^{14}C -tyrosine into total protein was measured. This amino acid was chosen because it is not metabolised in muscle and does not itself alter the rates of protein synthesis or degradation¹⁶. To determine absolute rates of synthesis from ^{14}C -tyrosine incorporated into protein, it was assumed that the intracellular tyrosine-specific activity reflected the tRNA precursor pool. This assumption is valid when high levels of tyrosine (0.5 mM) are added to the medium causing its specific activity to approach that of the intracellular pools¹⁸. When A23187 ($10\text{ }\mu\text{g ml}^{-1}$) was added to the medium, rates of total protein synthesis were increased by 44% (Table 1). This concentration of A23187 produced maximal stimulation of both protein synthesis and degradation. Addition of A23187 to muscles incubated in calcium-free medium did not affect protein synthesis, indicating a mode of action involving movement of extracellular Ca^{2+} into these cells.

Protein degradation was measured in two ways. In one method, net protein degradation was first determined as the amount of total tyrosine released with time into the muscle pools and medium¹⁶. The absence of metabolism of this amino acid means that the production of free tyrosine must represent the difference between proteolysis and protein synthesis. Addition of A23187 increased tyrosine release by 114% (Table 1). As protein synthesis is elevated in such conditions (Table 1), this increase in released tyrosine actually represents an underestimate of the absolute increase in the amount of protein degradation occurring. To determine rates of protein breakdown, the rates of synthesis and net degradation can be summed. This quantity increased by 71% on addition of the ionophore. An alternative method of measuring degradation involves the addition of cycloheximide to eliminate re-utilisation of amino acids¹⁶. Although cycloheximide may reduce absolute rates of degradation, the regulatory effects of various physiological factors on proteolysis can still be measured^{1,16}. In the presence of cycloheximide, A23187 stimulated tyrosine release by 43% (Table 1).

We then carried out experiments to characterise the increased protein breakdown caused by the Ca^{2+} ionophore. As in our experiments on protein synthesis, A23187 had no effect on rates of proteolysis in the absence of extracellular Ca^{2+} (Table 1), supporting a mechanism requiring movement of Ca^{2+} into the muscle cells. Interestingly, removal of extracellular Ca^{2+} by itself reduced degradation. To study the degradation which occurs with elevated intracellular Ca^{2+} , we tested the effect of leupeptin on proteolysis (Table 2). This non-toxic, highly specific protease inhibitor has been previously shown to retard muscle degeneration, proteolysis and myofibrillar disassembly without affecting protein synthesis^{19–21}. Addition of leupeptin inhibited the elevated rates of degradation which occurred in the presence of A23187 by 32% (– cycloheximide) and 21% (+ cycloheximide). A similar degree of inhibition was also seen in the absence of ionophore, as described previously²⁰.

Several studies have reported that protein synthesis and/or degradation in isolated muscles or muscle cells may be influenced by electrical stimulation and even passive stretch^{2,22–27}. As such processes also affect the levels of free intracellular Ca^{2+} , we investigated the relationship of tension to our ionophore experiments. Muscles were either incubated in a

Table 1 Effect of A23187 on protein synthesis and degradation in the presence (a) and absence (b) of calcium

Treatment	Protein synthesis (nmol Tyr incorporated per mg per h)		Net protein degradation (nmol Tyr released per mg per h)		Protein degradation (+cycloheximide) (nmol Tyr released per mg per h)	
<i>a</i>						
Control	0.186 ± 0.015		0.121 ± 0.008		0.182 ± 0.015	
+A23187	0.267 ± 0.006	+44%*	0.259 ± 0.015	+114%*	0.260 ± 0.004	+43%†
<i>b</i>						
Control	0.173 ± 0.009		0.084 ± 0.004		—	
+A23187	0.181 ± 0.015	+5% (NS)	0.082 ± 0.003	-2% (NS)	—	

a, Normal female rats (40–55 g) were killed by cervical dislocation and paired soleus muscles immediately removed with tendons intact and weighed. Each muscle was preincubated in the presence or absence of $10 \mu\text{g ml}^{-1}$ A23187 (Lilly) for 1 h at 37°C . The incubation medium (3.0 ml) also contained Krebs–Ringer bicarbonate solution saturated with 95% O_2 –5% CO_2 and supplemented with 10 mM glucose, 5× plasma levels of the branched chain amino acids (valine, isoleucine and leucine), and 0.1 unit ml^{-1} insulin¹⁶. A23187 was dissolved in ethanol and the final concentration of ethanol in all incubations was adjusted to 0.5% v/v. This amount of ethanol did not affect protein synthesis or degradation. After 1 h, the muscles were blotted and transferred to fresh medium of the same composition but containing in addition ^{14}C -tyrosine ($0.06 \mu\text{Ci ml}^{-1}$) and unlabelled tyrosine (0.5 mM). The muscles were incubated for 1 h at 37°C and then placed in 10% trichloroacetic acid and protein synthesis was measured as described previously¹⁶. Determination of synthesis was carried out by correcting rates of ^{14}C -tyrosine incorporation into acid-precipitable protein using the specific activity of the intracellular tyrosine pool¹⁶. For measurement of net protein degradation muscles were incubated without tyrosine to allow accurate measurement of the release of free tyrosine into the muscle pools and medium. Omission of tyrosine has no effect on rates of muscle protein synthesis or degradation¹⁶. After 1 h of preincubation the tyrosine content of the muscle pools was in a steady state in the presence or absence of ionophore; therefore, net protein degradation measured during the second hour was proportional to tyrosine appearing in the medium during that period. Tyrosine was measured fluorometrically as previously described¹⁶. Protein degradation was measured similarly except that cycloheximide (0.5 mM) was added to both the preincubation and incubation media. *b*, Muscles were treated as in *a* except that the Krebs–Ringer bicarbonate buffer was prepared without calcium but containing the calcium chelator EGTA (2 mM). Results are expressed as the mean value $\pm$ s.e.m., $n = 6$. *P* values were calculated for the paired muscle differences using the Student *t*-test. NS, not significant.

* $P < 0.005$. † $P < 0.003$.

flaccid state as in Tables 1 and 2 or were incubated with slight passive tension by pinning the tendons so the muscle was maintained at approximately 110% of rest length. When muscles were incubated under tension without ionophore, no effect on synthesis was detected (Table 3). Such results confirm earlier studies with the adult soleus which showed that active or passive tension did not increase synthesis in similar conditions^{2,25}. In contrast, fixing the length of the soleus in the presence of A23187 caused a further (23%) increase in protein synthesis above that caused by the ionophore alone (Table 3). Although passive tension alone had no effect on synthesis, as expected, the addition of A23187 to pinned muscles produced a large increase in synthesis (70%) equal to the sum of the above two effects (Table 3).

The increase in synthesis produced with A23187 may result from higher average intracellular Ca^{2+} levels than those which occur on intermittent stimulation or stretch as described previously^{2,25}. Stimulation may, however, produce higher transient Ca^{2+} increases, which would explain the greater peak tension produced with stimulation than A23187 in the soleus²⁸. Possibly, more frequent stimulation and/or stretch applied for longer periods further increases free Ca^{2+} and may account for several reports of increased protein synthesis caused by stimulation or stretch^{22–24,26}. In any case, the ability of A23187 to stimulate protein synthesis is seen when the muscles are flaccid, contracting against no load, indicating that the Ca^{2+} -dependent increase in synthesis does not require the development of tension. The further enhancement of synthesis seen when ionophore was added to muscles incubated with slight stretch may result from higher Ca^{2+} levels produced by longer sarcomere lengths²⁹ or possibly length-dependent effects on the sarcolemma. Related studies have shown that most of the Ca^{2+} -dependent protein synthesis requires RNA synthesis (G. Silver and J.D.E., in preparation).

In contrast to the potentiating effect of tension on Ca^{2+} -dependent synthesis, the effect of tension on degradation was opposite to that produced by A23187. Table 3 shows that maintaining muscle tension decreased rates of proteolysis in the presence of A23187. Furthermore, unlike the effect of tension on synthesis (which was only seen in the presence of ionophore), maintaining the muscle under slight stretch also produced a similar inhibition of degradation in the absence of A23187, as previously reported by Goldberg *et al.*^{2,25}. Although the mechanisms by which muscle stretch or activity reduce proteolysis are unclear, the present results argue against a Ca^{2+} -mediated effect. Further support for this conclusion comes from the observation that, even over a wide range of ionophore concentration, inhibition of degradation was not detected in the absence of tension (data not shown).

There are several possible mechanisms which can explain the

Ca^{2+} -dependent stimulation of proteolysis. Ca^{2+} may modify the structure of muscle proteins, rendering them more susceptible to proteolysis, or it may stimulate the activity of cellular proteases. Activation of proteolysis could involve an increase in the number of muscle lysosomes, leakage of lysosomal enzymes, or increased uptake of proteins into this organelle. Alternatively, mammalian cells are known to contain soluble neutral proteases^{30–33}, and Ca^{2+} -dependent protease(s) from muscle seem to be capable of initiating myofibrillar degradation and hydrolysing other proteins^{31–33}. Direct activation of such non-lysosomal proteases seems an attractive explanation of the ionophore effect on degradation rates, although other indirect mechanisms cannot be excluded.

The actual mechanisms involved in the Ca^{2+} -dependent effects described here are unclear, but several factors indicate that they do not involve gross toxic effects on cells. First, elevation of protein synthesis in the presence of the ionophore indicates good viability, as this process is sensitive to small drops in ATP levels. Also, exposure of the muscles to A23187 for 2 h produced no loss of subcellular structure or ability to produce maximum twitch tension on electrical stimulation (T. K., D. Erlij and J.D.E., in preparation). Finally, in previous studies with this muscle no alteration in resting potential was seen with the same concentrations of A23187 ($10 \mu\text{g ml}^{-1}$) producing maximal effects in the present studies²⁸. These results differ considerably from the results of certain workers using this ionophore in other systems. For example, studies with frog skeletal muscle have reported contraction accompanied by a decrease in resting potential and dramatic destruction of subcellular structure on exposure to A23187³⁴. Such effects may be related to our observations of decreased protein synthesis seen in certain muscle preparations or upon longer exposure to ionophore (data not shown).

Table 2 Effect of leupeptin on protein degradation in the absence (a) and presence (b) of A23187

Treatment	Net protein degradation (nmol Tyr released per mg per h)		Protein degradation (+cycloheximide) (nmol Tyr released per mg per h)	
<i>a</i>				
Control	0.139 ± 0.018		0.208 ± 0.011	
Leupeptin	0.073 ± 0.007	-47%*	0.162 ± 0.006	-22%*
<i>b</i>				
Control	0.181 ± 0.007		0.217 ± 0.009	
Leupeptin	0.123 ± 0.013	-32%†	0.171 ± 0.007	-21%‡

Muscles were incubated as described in Table 1. Cycloheximide (0.5 mM) and leupeptin (50 μM) were added to both the preincubation and incubation medium as indicated.

* $P < 0.01$.

† $P < 0.001$.

‡ $P < 0.005$.

Table 3 Effect of tension on protein synthesis and degradation

Treatment	Protein synthesis (nmol Tyr incorporated per mg per h)	Protein degradation (+cycloheximide) (nmol Tyr released per mg per h)
<i>a</i>		
Control	0.299 ± 0.034	0.275 ± 0.012
Stretch	0.286 ± 0.016	0.200 ± 0.011
<i>b</i>		
A23187	0.240 ± 0.007	0.417 ± 0.022
Stretch + A23187	0.295 ± 0.017	0.258 ± 0.021
<i>c</i>		
Stretch	0.243 ± 0.024	—
Stretch + A23187	0.412 ± 0.022	—

Muscles were maintained under tension throughout the preincubation and incubation periods by fixing the length of the tissues in a stretched position (110% of rest length) with pinning through the tendons into small plastic supports. Other muscles were incubated in a flaccid state. Ionophore ($10 \mu\text{g ml}^{-1}$) was added as indicated and measurements were carried out as described in Table 1. Differences between mean values for identical conditions tested during different experiments (*a*, *b*, *c*) illustrates day to day variations and the advantage of paired muscle analysis.

* $P < 0.025$.

† $P < 0.01$.

‡ $P < 0.003$.

In related studies we have found that combinations of different degrees of stretch and Ca^{2+} can produce effects consistent with muscle hypertrophy as well as atrophy (J.D.E., T.K. and K. Matsumoto, in preparation). However, the observations reported here closely resemble the hereditary muscular dystrophies. Thus, Ca^{2+} may be directly responsible for at least a substantial part of the increased protein turnover and net negative nitrogen balance characteristic of muscular dystrophies. Tension seems to be particularly effective in decreasing Ca^{2+} -induced net catabolism by increasing synthesis as well as decreasing degradation, and leupeptin seems able to decrease proteolysis in the ionophore-treated muscles. Thus, maintenance of passive tension, and other agents like leupeptin and possibly calcium chelators may be of therapeutic value in the treatment of certain muscular dystrophies.

We thank K. Kameyama and K. Matsumoto for assistance, L. Abott and R. Ginsberg for preparation of the manuscript, and D. A. Fischman for reading the manuscript. These studies were supported by the Commonwealth Foundation and by grants to J.D.E. from NIH (HL2197002) and the Muscular Dystrophy Association. Leupeptin was from the US-Japan Cooperative Cancer Research Program, and A23187 from R. Hamill (Lilly).

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Received 17 January; accepted 30 March 1979.

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Cloning and endonuclease mapping of the hepatitis B viral genome

THE virus that causes hepatitis B, or serum hepatitis, seems to infect only humans in nature, and experimental infection has been achieved in only a few additional mammals. The limited host range of the hepatitis B virus (HBV), and its failure so far to infect tissue culture cells have drastically restricted study of this virus and have hindered development of a vaccine for the serious disease that it causes. We report here the cloning of double-stranded HBV DNA in *Escherichia coli* K12, using the unique *EcoRI* cleavage site on the viral genome to introduce the entire HBV DNA molecule into an *EcoRI* cleavage site within the chloroamphenicol (Cm) resistance gene of the pACYC184 plasmid vector¹.

The Dane particle², a particulate form of viral antigen found in the blood of infected patients, seems to be the infectious form of HBV. DNA isolated from Dane particles has a molecular weight of 2.1×10^6 (corresponding to an approximate genome length of 3,200 base pairs), and is single-stranded for about 15–45% of its length^{3,4}. Using either the endogenous DNA polymerase of the Dane particle⁴ or avian myeloblastosis virus RNA-dependent DNA polymerase (reverse transcriptase)⁵, the hepatitis B viral genome can be converted to a double-stranded circular form, which is cleaved by the *EcoRI* endonuclease into a unit length linear structure (A. Siddiqui, F. R. Sattler and W. S. Robinson, in preparation). We have introduced this *EcoRI*-cleaved viral DNA into the *EcoRI* site of the Cm-resistance gene of plasmid pACYC184. The previously reported⁴ heterogeneity of Dane particle DNA has been investigated by analysis of multiple separately-cloned DNA molecules, and a restriction endonuclease cleavage map of the HBV genome has been constructed.

The surface antigen of the hepatitis B virus (HBsAg) is known to be antigenically complex; four major subtypes (adw, ayw, adr, ayr) have been identified, and recent immunological evidence suggests that antigenic heterogeneity exists also within each of these subtypes⁶. Siddiqui *et al.* (in preparation) have observed that Dane particle DNA isolated from carriers infected with different hepatitis B viral subtypes show divergent *HincII* and *HaeIII* endonuclease cleavage patterns, and some degree of heterogeneity has been found even within the same subtype. In order to circumvent heterogeneity between different HBsAg subtypes or between different carriers of the same subtype, the DNA used in our initial cloning experiments was isolated from Dane particles of a single HBsAg carrier (subtype adw). This method also allowed us to investigate further the question of possible heterogeneity of hepatitis B viral DNA isolated from a single carrier.

In order to decrease the frequency of recircularisation of the pACYC184 vector plasmid and to maximise formation of composite plasmids, *EcoRI*-cleaved pACYC184 DNA was treated with alkaline phosphatase⁷ before addition of *EcoRI*-

treated Dane particle DNA that had been made double-stranded using the endogenous DNA polymerase. The DNA species were ligated and introduced by transformation into a non-restricting (r_{K}^{mK}) mutant *E. coli* C600 (ref. 8); bacteria were plated on nutrient agar plates containing tetracycline (Tc, $10 \mu\text{g ml}^{-1}$) 40 min after the DNA uptake step of the transformation procedure to ensure that individual transformants

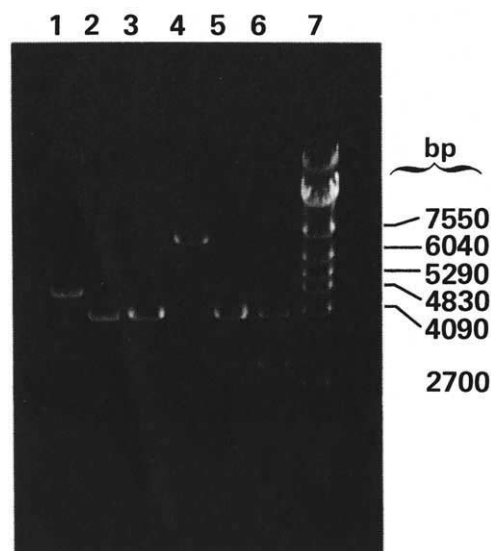


Fig. 1 Cloning and restriction endonuclease analysis of recombinants between Dane particle DNA and the pACYC184 plasmid vector. Dane particle DNA was isolated from the plasma of a chronic hepatitis B surface antigen (subtype adw) carrier 1083 and made fully double-stranded using the endogenous polymerase⁴. 100 ng of this DNA was digested with 0.5 units of *Eco*RI endonuclease (New England Biolabs) for 30 min as previously described⁸. pACYC184 DNA 2 μg isolated as described elsewhere¹² was simultaneously digested with a twofold excess of endonuclease *Eco*RI and treated with 0.2 U of calf intestine alkaline phosphatase (Boehringer) to prevent recircularisation during the subsequent ligation⁷. The reaction was stopped by heating at 65°C for 10 min, followed by phenol extraction. After precipitation by ethanol, the phosphatase-treated plasmid DNA was mixed with the *Eco*RI-cleaved fully double-stranded Dane particle DNA in a final volume of 50 μl , and ligated using 0.14 U of bacteriophage T4 DNA ligase (New England Biolabs) for 8 h at 12.5°C in standard conditions⁸. The reaction mixture was added directly to the CaCl_2 -treated *E. coli* C600 (r_{K}^{mK}) for transformation⁸. P3+EK1 containment conditions were used, as specified in the 22 December 1978 recombinant DNA guidelines of the US National Institutes of Health. Transformants were selected on Penassay base agar (antibiotic medium 2, Difco) containing Tc ($10 \mu\text{g ml}^{-1}$). After 24 h at 37°C , colonies were screened for insertions in the gene that codes for chloramphenicol resistance¹ by streaking clones on plates containing Cm ($25 \mu\text{g ml}^{-1}$). Plasmid DNA was isolated from those clones which were sensitive to Cm as described¹². Endonuclease digests of one representative plasmid designated pHBV1 were analysed by gel electrophoresis. Vertical agarose (0.7%, Seakem, M.C.I.) gels (20 cm long) were run at 35 mA (ref. 9). pACYC184 and pHBV1 DNAs were treated with the indicated endonucleases and molecular lengths were calculated using *Eco*RI-generated fragments of pRR12 as a size standard (see below) and are accurate within $\pm 10\%$ (ref. 13). Lanes: (1) pACYC184 DNA, showing uncleaved superhelical dimers and higher multimers; (2) pHBV1 recombinant DNA, showing uncleaved superhelical monomers and higher multimers; (3) *Hind*III-cleaved pACYC184 DNA, showing a single linear fragment (3.97 kilobases); (4) *Hind*III-cleaved pHBV1 recombinant DNA, showing linear fragment (7.91 kilobases), as HBV DNA is not cleaved by this enzyme; (5) *Eco*RI-cleaved pACYC184 DNA, showing single linear fragment (3.97 kilobases); (6) *Eco*RI-cleaved pHBV1 showing two fragments (3.97 and 3.3 kilobases); (7) *Eco*RI-generated fragments of plasmid pRR12 used as molecular length standards¹³. The lengths of the fragments shown are (from top to bottom) 20.39, 12.08, 11.40, 10.88, 7.55, 6.04, 5.29, 4.83, 4.09, 2.70, 1.66, 1.53 and 1.13 kilobases.

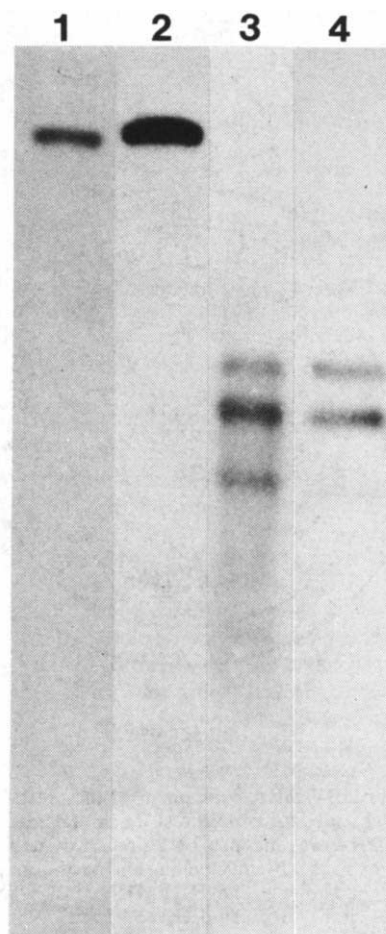


Fig. 2 Autoradiogram of pHBV1 DNA after endonuclease digestion and hybridisation with a nick-translated Dane particle DNA probe. The fractionation of endonuclease cleaved DNA was as described in Fig. 1, except that a 1.5% agarose gel was used. The DNA was transferred from the agarose slab gels onto nitrocellulose filters (Millipore) by the method of Southern¹⁴. After the transfer, the filter was incubated for 16 h in a $6\times\text{SSC}$ solution containing prehybridisation buffer (0.02% each of Ficoll, polyvinylpyrrolidone and bovine serum albumin)¹⁵. Dane particle DNA was labelled by nick translation^{16,17}. The specific activity of the DNA probe was 5.09×10^7 c.p.m. μg^{-1} . Initially, the ^{32}P -labelled DNA (1.5×10^6 c.p.m.) was denatured in a boiling water bath for 20 min in the presence of 1.3 mg ml^{-1} sonicated salmon sperm DNA (total volume 1.2 ml), chilled briefly, then brought to a final volume of 5 ml hybridisation buffer containing 50% formamide, $5\times\text{SSC}$, and 0.2% sodium lauryl sulphate. After removing the prehybridisation buffer, the filter ($8\times 15 \text{ cm}$) was annealed with the HBV probe for 12 h at 42°C in a sealed plastic bag. It was then washed twice for 1 h each time at 42°C with 25 ml of the hybridisation buffer and repeated an additional two times with the same solution but $0.5\times\text{SSC}$. The filter was finally rinsed with $100 \text{ ml } 2\times\text{SSC}$, air dried and exposed to an X-ray film in the presence of a Dupont Cronex intensifying screen at -70°C for 2 h. Lanes: (1) *Eco*RI-cleaved ^{32}P -labelled Dane particle DNA made double-stranded with endogenous polymerase, to serve as a marker for the location of unit length HBV DNA; (2) *Eco*RI-cleaved pHBV1 DNA; (3) ^{32}P -labelled nick-translated HBV DNA that has been cleaved with both *Eco*RI and *Hinc*II, to serve as a marker for the sizes of the endonuclease generated fragments from the genome; (4) pHBV1 DNA cleaved with both *Eco*RI and *Hinc*II. Lanes 1 and 3 were exposed to X-ray film before hybridisation was performed. The 94-base pair fragment resulting from the *Eco*RI cleavage of the *Hinc*II 'D' fragment (see Fig. 3) does not appear in the autoradiogram because it is not retained by this gel. The 220-base pair fragment resulting from this same cleavage of *Hinc*II fragment D does not appear in lane 4 because it is incompletely retained on the filters during hybridisation in the conditions used.

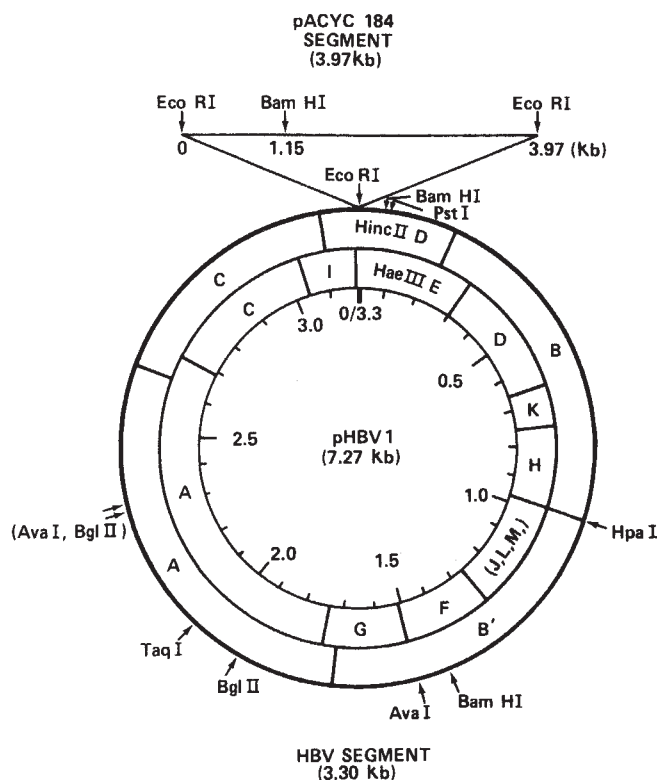


Fig. 3 Map of HBV DNA component of the pHBV1 plasmid, showing cleavage sites for certain restriction endonucleases. The cloned HBV DNA was mapped by a previously described procedure which involves ^{32}P -end labelling and partial enzyme digestions¹⁰ (data not shown). The HBV DNA insert contains no cleavage sites for the *HindIII*, *Sall*, *SmaI*, or *XhoI* endonucleases. The *EcoRI* cleavage site was arbitrarily chosen as the zero position for the map.

would represent separate clones. Tc-resistant transformants were selected and screened for resistance to Cm. Over 90% of those clones resistant to Tc were sensitive to Cm, indicating interruption of the continuity of the Cm resistance gene; plasmids from 25 of these were isolated and examined by agarose gel electrophoresis⁹ after *EcoRI* cleavage. Twenty of the cloned plasmids were found to contain a single DNA insert approximately 3,300 base pairs in length (Fig. 1); the remaining five plasmids had undergone deletion of vector DNA in the vicinity of the *EcoRI* cleavage site. Using *BamHI* endonuclease, which cleaves the pACYC184 and HBV DNA molecules asymmetrically, we found that both orientations of HBV DNA insertion were equally represented in these clones (data not shown).

The identity of the DNA insertions within pACYC184 was verified by cleavage of a selected composite plasmid (pHBV1) by several different site-specific endonucleases and by hybridisation of radioactively-labelled HBV DNA to the endonuclease-cleaved recombinant plasmid (Fig. 2). In addition, to facilitate further studies of the structure of the HBV genome and the proteins that it encodes, an endonuclease cleavage map (Fig. 3) of the pHBV1 plasmid was constructed using the procedure of Smith and Birnstein¹⁰. The location of endonuclease cleavage sites within cloned pHBV1 DNA is in agreement with a preliminary map (Siddiqui *et al.*, in preparation), constructed for HBV DNA isolated directly from Dane particles obtained from the same human carrier. This finding provides further verification of the nature of the DNA insert contained in the pHBV1 plasmid, and indicates also that the entire HBV genome is present and stably maintained in the recombinant plasmid. Experiments reported recently by Fritsch *et al.*¹¹ indicate that recombinants between HBV DNA and bacteriophage λ gtWES. λ B can also be propagated stably in *E. coli* K12.

Landers *et al.*⁴ reported that the sum of fragments produced by *HaeIII* endonuclease cleavage of Dane particle HBV DNA is greater than the molecular size of the entire virus, and concluded that the DNA molecules contained in different Dane molecules were not identical. In our studies of HBV DNA cloned in *E. coli* K12, we have observed that the sum of the sizes of the *HaeIII* fragments in 20 separate clones equals exactly the molecular weight of the virus. The difference between the fragmentation patterns observed by us for *HaeIII*-digested cloned HBV DNA, and the pattern reported by Landers *et al.*⁴ using Dane particle DNA obtained from a different carrier resides partly in the two largest *HaeIII*-generated fragments of the virus; *HaeIII* cleavage of the DNA studied by Landers *et al.* yielded a doublet with components estimated to be 986 and 957 base pairs. Electrophoresis of the cloned DNA showed a single fragment having a mobility consistent with a size of 986 base pairs, as did fully-stranded Dane particle DNA isolated from that carrier. Electrophoresis of a mixture of *HaeIII*-cleaved pHBV1 DNA with identically digested DNAs from 19 other HBV-pACYC184 recombinant plasmids we have studied showed no double band at the relevant location in the gel.

In addition, the cloned DNA does not show the 750-base pair *HaeIII*-generated fragment found in Dane particle DNA by Landers *et al.*⁴. Since this fragment appeared only in Dane particle DNA that was elongated with endogenous DNA polymerase in the presence of deoxyribonucleoside triphosphates, its presence may have resulted from incomplete 'fill in' of the single-stranded portion of the HBV genome.

The ability to clone the total genome of HBV in an *E. coli* K12 strain now allows the amplification and propagation of hepatitis viral DNA species isolated from individual carriers and the production of substantial quantities of HBV DNA for further structural studies. Investigation of endonuclease cleavage patterns of cloned Dane particle DNA derived from different carriers showing the same viral subtype, and from individuals infected with different subtypes, should elucidate the extent of heterogeneity of the HBV genome in such carriers and should enable correlation of DNA primary structure with subtype specificity.

In addition, the prospect of expression in bacterial cells of peptides encoded by the cloned HBV viral genome potentially provides a means of synthesising large amounts of viral surface antigen suitable for use in production of a vaccine for viral hepatitis.

These investigations were supported by grants from the NIH, the NSF and the American Cancer Society (ACS) to W.S.R. and S.N.C. J.J.S. is a postdoctoral fellow of the ACS.

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Received 13 March; accepted 6 April, 1979.

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matters arising

Improved amorphous semiconductors for solar cells

OVSHINSKY AND MADAN¹ have described the interesting results of incorporating fluorine in glow discharge deposited amorphous silicon (in particular a very low density of states within the gap.) However, as one would expect on general grounds, fluorinated material has a significantly larger energy gap than hydrogenated material. It therefore offers an even less good match to the solar spectrum, which would not improve the efficiency of solar cells made from it. Although the use of iodine rather than fluorine might be expected to be helpful in this respect it is still unlikely to reduce the energy gap to the near 1 eV value required for optimum spectral response. [The stability of the Si-I bond shown by the surface stabilisation of silicon by iodine², led me to discuss the possible use of iodine in glow discharge deposited silicon with several workers at the International Conference on the Physics of Semiconductors at Edinburgh in September 1979.]

During our group's early work on glow discharge deposition of amorphous materials (see refs 3, 4) it was observed that germanium and silicon-germanium alloys deposited by glow discharge from germane or germane-silane mixtures, even with rather small germanium content, show negligible photoconductivity compared with pure silicon. This would now be interpreted in terms of the Ge-H bond being weak compared with the Si-H bond, with the result that dangling bonds on germanium atoms are not stably hydrogenated in amorphous germanium and its alloys, and with the further consequence that these materials have a large density of states in the gap. It would therefore not be possible to prepare solar cells from them even though their absorption edge could be well matched to the solar spectrum. Clearly this situation would alter if a stronger bonding element, such as a halogen, were used to satisfy germanium dangling bonds. (Even the Ge-I bond is far more stable than Ge-H). What is suggested, then, is that particularly promising amorphous semiconductors for making efficient solar cells would be germanium and Ge-Si alloys incorporating halogen, the overall composition tailored to optimise the photoconductive response spectrum to solar radiation.

Note added in proof: The optimum energy gap for matching a cell to the AM1 solar spectrum is about 1.4 eV (see, for example, ref. 5). However, even at the Equator the Sun is not long at the zenith (implied by AM1), and the increasing atmospheric absorption at lower solar angles characteristic of most of the day (all of it at higher latitudes, outside the tropics) shifts the optimum energy gap to still lower values. It would only be with high temperature operation (above ~300 °C) that fluorinated amorphous silicon could be considered to provide a good spectral match. As the main aim in developing large area solar cells is to avoid concentration of radiation, the maximum temperature likely to be encountered with them is perhaps 65 °C.

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OVSHINSKY AND MADAN REPLY—Goodman suggests that (1) the optimal energy gap for solar photovoltaic energy conversion is 1 eV and (2) fluorinated amorphous silicon has a significantly larger energy gap than hydrogenated amorphous silicon, and thus concludes that amorphous germanium or Ge-Si alloys are preferential to amorphous silicon for solar-cell applications. Both of the above premises are incorrect.

It is well known^{1,2} that the optimal gap for solar photovoltaic energy conversion is about 1.6 eV, not 1 eV. For example, the maximum efficiency for Am-0 using a semiconductor with an energy gap of 1.6 eV is about 25%, more than half again as large as the 16% maximum efficiency of a material with a 1.0 eV gap³. Furthermore, the energy gap of our new amorphous Si:F:H alloy is determined by a Fowler plot and clearly stated in our letter⁴ is 1.65 eV, which is essentially the optimal value. In fact, it is not significantly different from the energy gaps of 1.55–1.8 eV obtained in silane-decomposed films^{5,6}, nor is it much larger than the 1.5 eV gap of nominally pure amorphous silicon^{7,8}. The reason for this is that the

fluorine concentration of the new alloy is <5% and thus the vast majority of the chemical bonds that determine the energy gap of the material are ordinary Si-Si covalent bonds.

The major purpose of our letter⁴ was to point out that the new alloy has many features in addition to its band gap that makes it more desirable than silane-decomposed material for solar-cell applications.

Note added in proof: Goodman in his note in proof now suggests that higher air-mass conditions would greatly deteriorate the performance of amorphous Si:F:H solar cells on the basis of a 1.65-eV gap. The facts are as follows⁹. At AM-2, when the sunlight is at an angle of 60° to the Earth's surface (approximately the spectrum for average, slightly hazy weather conditions and smaller sun angles, as in temperate-zone climates), the maximum conversion efficiency of an ordinary solar cell is ~26%. For a gap of 1.65 eV, the maximum conversion efficiency is still 25%. This figure, in fact, is larger than the maximum conversion efficiency at any energy gap for AM-0 conditions (because of the decrease in relative solar irradiance in the near infrared at AM-2). The main point is that the maximum conversion efficiency is only a weak function of energy gap in the 1.0–2.0 eV range (never dropping below 20% in this range at AM-2), so that the efficiencies of real devices are much more dependent on other parameters.

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Asbestos-enhanced uptake of carcinogens

THE results of Lakowicz and Hylden's studies¹ on asbestos-mediated uptake of benzo(a)pyrene (BP) by dipalmitoyl L- α -phosphatidylcholine (DPPC) vesicles are more indicative of *in vivo* transfer of BP from particulates to lung surfactant (mainly DPPC and other phospholipids²)

than transfer of BP from particulates to biological membranes (mainly phospholipids and globular proteins³). As a monomolecular layer of surfactant containing DPPC exists at the air-liquid interface in the lung, BP adsorbed onto inhaled particulates would be at least partially transferred to lung surfactant before transfer to cell membranes would be initiated.

I suggest that the reported¹ increased rate of transfer for BP adsorbed to particulate silica or amosite asbestos as compared with BP in a microcrystalline form is principally a result of the adsorption of the mobile DPPC vesicles onto the particulate surface, rather than increased solubilisation of BP in an aqueous phase. It has been shown that DPPC readily adsorbs onto the surface of glass fibres and various types of asbestos, including amosite⁴. The adsorption is controlled by coulombic interactions between the ionic head group of the DPPC and the electrostatic charge of the particulates. The particulate surface charge also is significant in determining particulate-membrane interactions⁴. While the particulate surface charge controls DPPC adsorption, the particulate specific surface area and the form of the adsorbed BP (such as microcrystalline or monomeric¹) determine the surface area covered with BP at the particulate-liquid interface and thus the availability of BP for transfer to adsorbed DPPC or cell membranes. The interfacial availability of BP would govern relative transfer rates for particulates, such as silica and amosite, which display essentially the same surface charge⁴. On the basis of these comments, I suggest that the preferential interfacial transfer of carcinogens to cell membranes and/or lung surfactant at the particulate surface may be an important factor in co-carcinogenesis.

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LAKOWICZ ET AL. REPLY—we are aware that DPPC is a major component of lung surfactant; however, it is not known whether carcinogens which are adsorbed to particles are eluted during contact with surfactant or after subsequent contact with cells. For this reason we are investigating the effects of asbestos and silica on uptake of BP into natural membranes, namely rat liver microsomes and 3T3 cells. Again we find that these particles enhance BP uptake and that asbestos is

more effective than silica. Thus, it is clear that the particle-enhanced uptake of BP is a more general phenomenon which could occur not only in lung surfactant but also in lung cells.

The mechanism of the transfer process has not been elucidated. We suggested that the particle enhancement of uptake results from an increased rate of solubilisation of the BP in the aqueous medium, whereas Light proposes that the transfer of the BP is to vesicles which have become bound to the particulates. The basis for our argument comes from more detailed studies of the particle-enhanced uptake of another polynuclear aromatic hydrocarbon (PAH), 1,2-benzanthracene, into DPPC vesicles¹. In that instance we measured virtually the same uptake kinetics over a wide range of particle to DPPC ratios. For this reason we propose that the enhanced uptake results from an increased rate of solubilisation of these PAH by virtue of their association with the particulate materials. We do not intend to indicate that the particles can increase the equilibrium solubility of the PAH.

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The evidence for species guilds is an artefact

THE evidence cited by McNaughton¹ that ecosystems are loosely linked sets of species guilds seems to be based on an unfortunate artefact. Although the independent occurrence of species in samples is appropriately tested using the point correlation coefficient (V), it is effectively precluded by the sampling method used. This leads to a non-zero expectation for the point correlation coefficient for species whose spatial distributions are independent.

The dependence introduced by the sampling method may be demonstrated analytically. If an area occupied by a number of species distributed independently in space is sampled and the resulting distributions of the a th and b th species (A and B) among samples are independent, then

$$P\{A \cap B\} = P\{A\}P\{B\} \quad (1)$$

Where the events A and B are the occurrence in a sample of A and B , respectively. However, if it is sampled in the manner described by McNaughton it can be shown that

$$P\{A \cap B\} = P\{A\}P\{B\} - (1 + (k-1) \times (h+1) + k^2 g_a g_b) f_a f_b / (g_a g_b) \quad (2)$$

In this, f_i is the proportionate cover of the i th species, $g_i = 1 - f_i$, and, summing over all species except A and B , $h = \sum_i f_i$ and $k = \sum_i f_i / g_i$. As $g_i \leq 1$ for all i , $k \geq h$, so that equation (2) implies

$$P\{A \cap B\} \leq P\{A\}P\{B\} - h^2(1 + g_a g_b) f_a f_b / (g_a g_b)$$

Hence, as long as A and B occur, together with at least one other species (that is $0 < P\{A \cap B\} < 1$),

$$P\{A \cap B\} < P\{A\}P\{B\}$$

This contradicts equation (1). It follows that if two species independently distributed in space are sampled by this method and a point correlation can be calculated between them, its expected value is negative.

Due to its dependence on k , this bias declines with increased numbers of species as long as the form of the species abundance curve remains consistent. To determine the extent to which this might account for the effects observed by McNaughton, the sampling method was simulated on a computer, the first individual of each sample being selected at random from the species complement, the second at random from all species except that of the first. The average interaction term i and connectance c were estimated for sets of 100 sample points in the manner used by McNaughton, calculating V only when both species were represented by at least 10 individuals.

The extent of the bias in V depends on the frequency distribution of species abundance. It was postulated that the ranked population frequencies of the

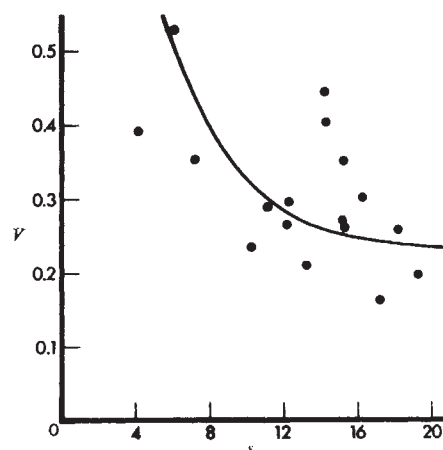


Fig. 1 The relationship between average interaction strength and number of species generated by the use of the sampling method of McNaughton¹ on species distributed independently in communities with log-series species abundance curves (solid line) for comparison with that reported for African grasslands.

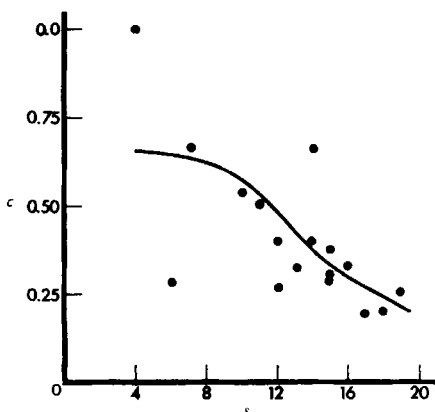


Fig. 2 The relationship between connectance and species number generated with the sampling method of McNaughton from communities with log-series species abundance curves for species distributed independently in space (solid line) for comparison with that reported for African grasslands.

species sampled formed a geometric series, with common ratio $\exp(-1/\alpha)$. Random sampling from such a distribution yields a log-series species abundance curve with diversity index² α . Such curves have frequently been found to provide an adequate description of species abundance data, albeit largely for insects², and have the advantage that they are effectively completely determined by sample size and species number.

Mean values of species number (s), $\hat{i}$ and $\hat{c}$ were determined for various values of α . The relations of $\hat{i}$ and $\hat{c}$ to s thus obtained are superimposed on the data of McNaughton in Figs 1 and 2. The curves seem to fit the data points adequately, leaving no residual systematic effect, affirming that the apparent decline in these parameters with increased species number is an artefact of the sampling method used.

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Stability and diversity in grassland communities

IN his analysis of the stability properties of large, randomly constructed model ecosystems, May¹ contradicted the existing ecological dogma—that increased community complexity produces increased stability—by showing that in Lotka–Volterra models, dynamic stability is diminished with increases in: (1) the number of species (S); (2) the average strength of the interactions among species (i); (3) the connectance of the system (c), that is, the proportion of non-zero values for i in the community interaction matrix.

He showed that the system is almost certainly stable if

$$i(Sc)^{1/2} < 1 \quad (1)$$

McNaughton² uses data from a study of the interactions among plant species in 17 grassland stands in the Serengeti National Park to test May's results, and concludes that both average strength of interaction and connectance are negatively correlated with species richness, implying that the structure of these grassland communities is constrained in special ways to meet the requirements for local stability. If this were true, it would be an important conclusion³. Unfortunately, McNaughton's results are an inevitable consequence of varying S , and hence say nothing about whether interactions in these communities are constrained by dynamic interactions between species.

McNaughton measures the strength of interaction among the plant species by applying to nearest-neighbour data⁴ the point correlation coefficient, V (giving limits of $+1$ if the two species always occur

we hope that further analysis of data of this kind will now be attempted by others.

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MCNAUGHTON REPLIES—Lawton and Rallison's statement that "as species richness increases, V is bound to decrease" is incorrectly applied to my Letter. As I reported negative values for V , the number of contacts would have increased, not decreased, had my initial report been correct. But it was wrong and Harris accurately identified the flaw.

I am grateful to them for calling attention to my error and hope that other ecologists using 2×2 contingency tests in this type of analysis will not make my mistake, whether using V or other statistics, such as χ^2 . I should have calculated an expected V , based on the species' relative abundance, and then used the t -test based on the z -transformation to determine whether the calculated V departed from the expected value. It is, therefore, of value to other ecologists who use nearest-neighbour analysis, or similar methods based on contingency testing, to have the expected values of the cells.

Using the traditional notation of a, b, c, d , for the cells of the 2×2 table, expected values for all sampling events consisting of two consecutive draws with replacement are, if the individuals are randomly arranged, $a = 2f_x f_y$, $b = f_x^2 + 2f_x f_y$, $c = f_y^2 + 2f_y f_x$, and $d = h^2$, where $f_x = n_x/N$, $f_y = n_y/N$, and $h = 1 - f_x - f_y$.

This obviously results in an expected negative value of V that increases in magnitude as f_x and f_y increase. But it also allows a straightforward test of spatial associations comparing observed and expected values of V . If, on the other hand, nearest neighbours of the same species are classified as 'pseudospecies', one traditional method in nearest-neighbour analyses, f_x^2 and f_y^2 are removed from the b and c cells and added to the d cell, resulting in expected positive values of V for randomly distributed individuals.

Another alternative previously used in nearest-neighbour analyses of vegetation is to classify intraspecific nearest neighbours as 'no contacts', eliminating them from the analysis. But then each cell must be corrected by $1 - f_x^2 - f_y^2 = m$, which apparently has not been done, and $a = 2f_x f_y/m$, $b = 2f_x h/m$, $c = 2f_y h/m$, and $d = h^2/m$. Finally, I redrew (selected the next nearest neighbour of another species) when nearest neighbours were members of the same species. To my chagrin, the

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

together, -1 if they never do, and a value of 0 if the species are distributed independently⁵, and shows that V is negatively correlated with S . However, he seems to make no allowance for the fact that, as species richness increases, V is bound to decrease. Harris presents the argument in full.

Finally, McNaughton² draws attention to another theoretical possibility, suggested by May¹, which also enhances the probability of stability in ecological systems; namely, that communities are organised into blocks of species. McNaughton suggests that the size of these blocks (or guilds) can be estimated by Sc . Unfortunately, Sc says nothing about whether the connections between species are 'blocked' and cannot measure the size of these blocks, even if they exist. Blocking depends on the arrangement of the interactions between species, and not on how many there are.

However, we agree with McNaughton that it is important to attempt to test stimulating ecological theories in the field, and

expected value of V with this approach also is negatively biased with

$$a = 2f_x f_y + f_x^2 (f_y/1 - f_x) + f_y^2 (f_x/1 - f_y)$$

$$b = 2f_x h + f_x^2 (h/1 - f_x) + \sum_{i=1}^s [f_i^2 (f_x/1 - f_i)]$$

$$c = 2f_y h + f_y^2 (h/1 - f_y) + \sum_{i=1}^s [f_i^2 (f_y/1 - f_i)]$$

and

$$d = h^2 - \sum_{i=1}^s [f_i^2 (f_x/1 - f_i)] - \sum_{i=1}^s [f_i^2 (f_y/1 - f_i)],$$

where f_i is n_i/N for each of the s species other than x and y . When I reanalysed my data, using the t -test as described above, the negative correlations I reported disappeared completely, as Harris predicted.

References 13–15, 18 and 19 in my initial Letter, as well as other sources², can be consulted for abundant evidence justifying my assumption that the spatial arrangements of individuals in local areas may reveal the nature of interactions among them. Naturally, experimentation would be the next step.

I thank W. T. Starmer for taking me 'back to basics', showing me two of the possible ways of treating intraspecific nearest neighbours and pointing me in the direction of others.

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Mutagenic effect of aromatic epoxy resins

ANDERSEN ET AL.¹ state that 'the demonstration of a mutagenic effect of aromatic epoxy resins (that is, those based on bisphenol acetone, BPA) indicates a genetic hazard, including a cancer risk, for humans exposed to these compounds'. We challenge the rationale for this rather definite conclusion. Epoxides as a class are reactive chemicals and in general are alkylating agents. However, in the extrapolation from direct alkylation of DNA to defining the risk of mammalian mutagenicity or carcinogenicity, other major factors must be considered, in particular the dose reaching target tissues/molecules and the importance of the detoxication or intoxication mechanisms which are only properly developed in the *in vivo* situation.

Such considerations are being included in a series of mutagenic assays to test for possible mammalian genotoxicity^{2–5}. But at present, *in vivo* studies must be con-

sidered as giving more definitive data than work carried out *in vitro* or in non-mammalian systems.

It is surprising, therefore, when a BPA-based epoxy resin which has been tested several times for carcinogenic potential with negative results is now considered to be a potential carcinogen based on the results of a microbial mutagenicity assay (although it does indicate that *in vivo* mutagenicity studies are required). Andersen *et al.*¹ review some of the animal data, but we would summarise the available cancer studies as follows:

(1) Andersen *et al.* do not critically evaluate the paper by Kotin and Falk⁶. The latter do not provide sufficient experimental details, in particular the route and frequency of exposure, to enable any conclusion to be drawn from their work, but it can be stated that there was definitely more than one exposure to the test material.

(2) The results of Weil *et al.*⁷ are not correctly interpreted by Andersen *et al.* They carried out two skin painting experiments. In the first, probably 30 mice were used and one papilloma was noted. In a repeat study, probably using 40 mice, no skin tumours occurred. The mortality of these mice was quite normal; there are no grounds for stating that the study was inadequate as the mice were dead in less than 24 months—the mice used by this laboratory lived their normal life span as evidenced by Weil's other data, and an average life span of about 18 months in mice is quite acceptable⁸.

(3) Hine *et al.*⁹ carried out a skin painting study, not referred to by Andersen *et al.*, in which a typical BPA-based epoxy resin was tested in both mice and rabbits without any skin tumours developing. Further, reference by Andersen *et al.* to the injection site sarcoma data only must be questioned as these tumours are not generally accepted as providing any reasonable indication of carcinogenic hazard (for example, gold and hypertonic saline were shown to be carcinogenic by this route^{10,11}).

In conclusion, the preponderance of available evidence indicates that currently used BPA-based resins present no carcinogenic hazard to man. This assessment is not changed by the evidence of Andersen *et al.*, although their findings should definitely stimulate further mutagenic studies.

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ANDERSEN ET AL. REPLY—Granville challenges our conclusions based on positive Ames tests with aromatic epoxy resins (AER). He claims that our conclusions are too definite and argues that tests in mammals would much better predict the risk for mutagenic and carcinogenic activity in humans. Conventional animal tests use only about 200 animals and are, therefore, rather insensitive. Only strong carcinogens will be identified by such tests^{1,2}.

The animal tests that were made on AER in the 1950s and early 1960s are inconclusive. The tests which did not show tumours are faulty and insufficient. In several cases, exact information on the experimental details is lacking. Only about 100 mice and 50 rabbits were used^{3,4}. The rabbit experiment was, according to the authors themselves, rather insensitive. Nevertheless, Granville claims that the experiments indicate that AER does not represent a cancer hazard, despite the experiment—albeit another one suffering from deficiencies in the description of the test conditions—in which tumours were produced by the resins⁵.

Thus our strong suspicions of dangerous effects of AER are in no way disproved by existing animal tests, nor will any new animal carcinogenicity test be able to give definite proof for non-carcinogenicity. It is our opinion that the doubt which might exist about the mutagenic or carcinogenic activity of a chemical substance should be used for the benefit of people who will come in contact with the substance. The only clear proof that AER is not dangerous to humans could come from experiments proving that AER cannot reach the relevant target molecules, namely DNA. As long as these experiments have not been made, AERs must be considered as mutagens and carcinogens.

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reviews

Exciting endocrinology

J. A. Parsons

Pioneers in Neuroendocrinology, Vol. II. Edited by J. Meites, B. T. Donovan and S. M. McCann. (Plenum: New York and London, 1978.) £20.48.

THIS fascinating collection of micro-autobiographies will delight those who already know that scientists as a group are neither more nor less often inspired, neither more perfect nor more perverse than others who make a living by their wits. It will also be received with joy by those who doubt the second of both these pairs of propositions. There is enough in it to reinforce almost any prejudice and material for many an after-dinner story.

Two quotations may whet the appetite and show the potential of the book in arguments for or against the 'Two Culture' hypothesis.

"Harmony in science respects the same type of stringent rules that can be found in Bach's polyphony . . . It really does not matter, for the progress of science, whether you are personally exploiting one of your previous findings and working on the next discovery, or whether the next step is taken by others who have picked up your idea. Some people feel this is stealing from you. In my opinion, it is the best compliment one scientist can pay another".

"My one application to Jefferson Medical College was rejected, to my parents' chagrin but to my delight, and for the next two years I worked as a professional musician in the Philadelphia area".

Inevitably in a book of this type, the contrast of personal philosophies is as remarkable as the difference in literary styles, but most contributions are penetratingly self-critical and many are full of humour.

"Du Vigneaud once told me that in the isolation of oxytocin, the most efficient purification step had been that of separating the pituitary from the cow".

Also, at a time when the number of young people of exceptional ability who choose scientific research as a career seems to be falling, one value of such a collection of honest attempts 'to tell it like it is' may be to provide an insight into the challenges and rewards of this way of life, far more vivid than any description from a careers officer.

Contributions to this volume were of course only invited from those whose work has succeeded, in the sense of making important contributions to a

relatively new branch of endocrinology. Another way in which its publication can be of general value is therefore by allowing some comparison of research careers and of the contributions made by different types of funding.

As one would expect in our era, the overwhelming majority of the work has received state support. The total of 24 contributors is not a large statistical sample, but it is noteworthy that 20 worked with grants in a university environment. Of the remainder, two were on the staffs of privately endowed research institutes (though they, too, received much state funding), two carried out some of their work in government research institutes, and one was supported principally by the US Veterans Administration.

As it can be predicted with some confidence that during the next decade neuroendocrinology will make major contributions to drug treatment of disease, it is again of some interest that 14 of the contributors had their formal training in physiology and/or anatomy, four in zoology, two in psychology and only two in pharmacology. Only five mention industrial support for some of their work. No less than 12 of the authors were trained as physicians before specialising in a basic science, a career pattern which has now become very rare in the UK.

The editors evidently had no distaste for controversy and several spectacular old battles receive an airing, with much detail of intellectual arguments and strategy and at least one allegation of foul play. The history of science in the making is clearly fraught with the same problems as those encountered in histories of the more distant past. As usual, the details of such accounts are of greatest interest to the participants and those responsible for funding them, but it seems worth drawing attention to one issue of general significance.

Major scientific journals exercise great power, and the forces of competition tend to ensure that their editors exercise this responsibly in maintaining the quality of work accepted. However, an incident which is discussed at some length raises the question whether editors are equally conscious of the responsibility incurred in rejecting a paper. Many will agree that the regret-

table practice of anonymous rejection by the use of a supply of forms is too common; justice and the public interest seem to require that dismissal of any carefully presented paper should be based on equally serious scientific assessment. Not only may ill-considered rejection delay general awareness of work later recognised as important, perhaps by several years if authors are diffident; as illustrated by the incident discussed, still worse may ensue if the rejection is based on comments of a single carelessly-chosen reviewer, who has thus received privileged communication of the results (most dangerous of all if he is a competitor). Justice is usually done in the end—but at what cost?

Quite apart from the human interest, the book is worth reading for its excellent science. For example, there are some of the clearest discussions one could wish of the (unfinished) list of frustrations encountered in trying to isolate the Corticotrophin Releasing Factor. There are also valuable insights into the role of the hypophysial portal circulation, presented notably by Bogdanove and Halász. Incidentally, although its relevance is not directly discussed, this evidence for highly directional transfer rather than simple diffusion of hypothalamic factors should surely lead to an armistice in the old battle over functional significance of the portal vessels. (The argument is here pursued unrelentingly by Lord Zuckerman himself, while Harris' perceptions are ably defended by Dr Donovan.)

In short, the editors are much to be congratulated for their enterprise and for showing clearly how much further excitement neuroendocrinology is likely to provide in the coming years. Perhaps we can look forward to another volume when such important issues are resolved as the structures of CRF, GRF and MIF, the role of prolactin in the origin and development of mammary tumours (interestingly discussed by Meites), and the probable physiological role of centrally acting peptides affecting memory and behaviour (excellently reviewed by de Wied). □

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Planetary atmospheres

Theory of Planetary Atmospheres: An Introduction to their Physics and Chemistry. By J. W. Chamberlain. Pp. 330. (Academic: New York, San Francisco and London, 1978.) £19.15.

It is a brave person who considers writing a textbook on planetary atmospheres at this time when there are so many space missions to the planets rapidly advancing our knowledge of these bodies. One procedure is to concentrate on basic processes and then use the current planetary observations to illustrate the mechanisms involved. Basically, this is the approach adopted by Professor Chamberlain whose valuable textbook is primarily concerned with the physical and chemical properties of planetary atmospheres. The author has made good of our effluent society, which in the past few years has produced detailed investigations of photochemical processes related to the potential climatic impact of variations in the Earth's ozone layers. This has allowed the discussions to be placed on a moderately firm foundation before their application to the planets.

The book covers approximately 300 pages of text supplemented by some useful appendices. Each of the seven chapters has a valuable list of references, that may be used to extract more detailed analyses of the work presented. In addition, there are problems at the end of chapters that make the text useful as a teaching aid for the rapidly increasing number of courses in planetary studies at universities throughout the world.

The introductory chapter discusses the basic physical concepts and provides the reader with a good background to the vertical structure of the Earth's atmosphere, together with an indication of the differences of structures of the other planetary atmospheres. I thought that the comments on p40 regarding the initial interpretation of the Pioneer 10/11 results for the structure of the upper atmosphere were unnecessary. The problem first identified when reducing these measurements has been resolved. Furthermore, the Voyager observations had now added further important information that indicates interesting structure in the upper Jovian atmosphere which now means that part of Figure 1.19 is out of date. I had hoped that Professor Chamberlain would not have introduced the term "dust" on p38 when discussing the ultraviolet absorbing layer created from photochemical products thought to reside above the visible clouds. This is an unfortunate term, misused for too long.

The introduction to atmospheric motions is well presented, although some aspects of the planetary examples are a little sketchy. In particular the reader still would have no idea why Jupiter (and Saturn too) have cloud bands from the discussions presented here. With many aspects of the Jovian dynamics still to be resolved, it would probably have been better to have shown how spacecraft measurements that could help resolve the controversial issues that currently exist.

The next two chapters are concerned with the chemistry and dynamics of the Earth's stratosphere and planetary astronomy where the basic concepts of radiative transfer are discussed. Professor Chamberlain is well known for his work in these areas, and the work is well presented. I still maintain, from a personal viewpoint, that the classical Chandrasekhar approach to radiative transfer is not the easiest way to introduce the subject to the reader. How-

ever, the references at the end of the chapter contain papers with other approaches, so the reader can decide this matter for himself.

The remaining chapters are concerned with ionospheres, airglows and aeronomy and the stability of planetary atmospheres. This indicates the breath of the discussions which with a good mathematical description form the basis of a useful book. I personally consider this volume a valuable addition to the subject. I sincerely hope, therefore, that the publishers will have the wisdom to produce an inexpensive paperback version so that students attending courses in this rapidly growing subject will be able to purchase a copy of their own.

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Guide for Darwin scholars

Charles Darwin: A Companion. By R. B. Freeman. Pp. 309. (Dawson: Folkestone, UK; Archon Books/Shoe String Press: Hamden, Connecticut, 1979.) £12.50; \$27.50.

RICHARD FREEMAN is rightly held in high regard for his meticulous labours on the bibliography of Charles Darwin's writings. His equally significant work *Charles Darwin: A Companion* is likely to prove indispensable to Darwin scholars. Interest in Darwin continues to grow, as continued exposure on the media attests and the steady flow of learned publications sustains. Recent discoveries are deftly and economically incorporated with notable conciseness.

There is a clear need for an enumeration of securely determined facts about Darwin: about his family from the sixteenth century record in Lincolnshire to his grandchildren; about the servants, dogs and horses as well as those of their close friends; about the names and ever baffling nicknames of their ramifying and closely intermarrying relatives; and about the Hookers, Huxleys, Henslows and Wedgwoods.

The *Companion* is an alphabetical list of names; wives are listed under their maiden name with a cross reference to their husband. All cross-references are cunningly devised to encourage wandering as should always be the case in a good work of reference. Quotations from Darwin enliven the identity and relevance of each name so that the book becomes a truly

fascinating anthology of quotations as well as a detailed guide to the formidable bibliography. The forty-one pages of material devoted to Charles Darwin required 25 sub-headings listed on page 71. The sub-headings are set in an insufficiently bold type and it would have been helpful to the reader to carry the sub-heading at the top of the relevant page. Particularly worthy of note are the "few quotations to give indications of CD's character" (pages 71-2) which include new discoveries amongst old friends. In his introduction Freeman quotes Darwin's letter to Huxley from Ilkley in November 1859 (LLii281): "The difficulty is to know what to trust." when making a compilation from various sources. Richard Freeman, an accurate compiler with a discerning eye for the unusual, is sadly let down by careless transcriptions in the printed texts. Both Francis Darwin and his sister Henrietta placed unjustified faith in the transcriptions they published. Hence, we read instead of Moscheles as teacher of pianoforte to their mother the name Maschelas, and Henrietta transcribes the name of Darwin's amanuensis and companion Syms Covington as Conington (Emma Darwin ii 19). It was a more recent misreading by a dealer in manuscript letters which resulted in the entry or page 167 for Hoskins an untraceable botanist. The original of this letter to Henslow has since come to light and reveals that CD wrote Hooker, indeed a botanist and in 1845 an unsuccessful candidate for the Chair of Botany at Edinburgh.

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Theory of conditioning

Classical Conditioning and Operant Conditioning: A Response Pattern Analysis. By W. W. Henton and I. H. Iversen. Pp. 355. (Springer: New York, Heidelberg and Berlin, 1978.) DM 54; \$29.70.

THE argument of this book is that the phenomena of conditioning can best be understood by observing behaviour in all possible detail, and describing the effects of contingencies between stimuli, rewards and responses on everything the subject is doing. The authors build up an impressive, if partisan, case for this "response pattern" theory and for the "multiple response" experimental technique which it implies. Along with these latter, however, they bring us a metatheoretical commitment to a hard-line Skinnerism, according to which even the editorial policy of the *Journal of the Experimental Analysis of Behavior* is dangerously revisionist. Any attempt to use behaviour as an index of an underlying state, physiological or mental, is consistently condemned as "phrenology".

This package of experimental method, theory and ideology is applied to five problems: classical conditioning against an instrumental baseline (conditioned suppression and its variants); concurrent operant performances; multiple schedules of reinforcement; collateral (adjunctive) behaviours; and concurrent classical conditioning. In each area the authors produce original experimental ideas and have provocative remarks to make about currently dominant theories, and they repeatedly show that if the attempt is made to record all an animal's behaviour, regularities of response sequencing can be found to underlie effects of reinforcement schedules that have mainly been analysed at a more molar level in the past.

Although Henton and Iversen break away from recording a single "arbitrary operant" response, they still use arbitrary subjects (rat, pigeon, monkey) in wholly artificial, stereotyped learning situations. But it is a little unfair to criticise them for faults they share with most of operant psychology. More seriously, they entirely ignore the regulatory aspect of instrumental responding, which must constrain the subject towards that "averaging over minutes, hours or days" which the authors condemn. In fact they never ask themselves either what the behaviour they observe so microscopically, or the conditioning they are trying to explain, might be for. I cannot help feeling that this blindspot, which greatly weakens

the authors' argument, is partly due to their ideological position.

Minor niggles include the lack of an author index (exacerbated by having separate bibliographies for each chapter), a high misprint rate, a slightly petulant tone savouring of "Reply to Reviewer B" in places, and occasional lapses from English idiom, which, though never a threat to understanding and also forgivable from Iversen, ought to have been caught by his publisher or co-author. There are two serious omissions from the point of view of the reader's convenience. First, the authors miss the opportunity to establish a consistent way of presenting multiresponse data. No book containing detailed accounts of 31 experiments can make easy reading, but repeated changes in

diagram conventions make things harder than they need be. Secondly, we could have done with summary tables, for each chapter or the book as a whole, giving the main procedures and results of all the experiments. As it is, there isn't even a concluding chapter to review and summarise the argument. Nevertheless, the book deserves a welcome, partly for giving an integrated account of an extended research programme, but mainly as an empirical challenge to some widespread current generalisations about conditioning.

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Sensory integration

Handbook of Behavioral Neurobiology. Vol. 1: Sensory Integration. Edited by R. B. Masterton. Pp. 579. (Plenum: New York and London, 1978.) £24.88.

IN the words of the publisher *The Handbook of Behavioral Neurobiology* will provide "a critical systematic enquiry into those aspects of neuroscience having the most direct and immediate bearing on overt behaviour." The targets for this first volume are "practising neuroscientists looking for a concise and authoritative treatment of developments outside their particular specialisation, and students who need an overview of the persistent and current problems surrounding the relation of the perceptual systems to behaviour". The eighteen authors were asked to "sacrifice comprehensiveness for illumination". Without exception they have complied with editorial pressure and in so doing have produced a surprisingly well integrated book which should achieve its aims.

The ten chapters dealing with the individual sensory systems form the core of the book. Not surprisingly the visual system claims more space than any of the others, but it is closely followed by the vestibular and auditory systems. Olfaction, in some ways the most interesting of the senses, brings up the rear with less than half the space earned by the gustatory and somatosensory systems. The reader, be he a practising neuroscientist or a student, will probably first turn to one of these chapters; and he will not be disappointed. Without exception the authors have provided illumination and not always at the expense of comprehensiveness.

It would be a pity, however, if the first three integrating chapters escaped attention. Erickson who writes chapter three prefaces it with a quotation from Poincaré: "Science is built up with facts as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house". These first three chapters provide the reader with plans for a house. Erickson himself argues that our knowledge of sensory processes will be constrained "if we resist seeing beyond the idiosyncracies of each system". He then moves towards a general theory of sensory neural function. In the second chapter, Cunningham and Murphy explore the ontogeny of sensory systems and ask whether their developing structure and function are wholly genetically determined. Despite the weight of the present evidence they think not, and predict that future work using anatomical and physiological techniques which can detect the subtleties of sensory perception will show that the contribution of nurture to sensory development is the rule rather than the exception.

Finally the editor's opening chapter surveys the evolutionary history of the six sensory systems. For each he provides a useful common plan for all vertebrates and then briefly discusses any striking variations. He concludes each section with a discussion of the notable changes which have probably occurred in the evolution of the human sensory system in question.

These three chapters provide a good introduction to a useful book which sets a high standard for those which are to follow.

J. R. Symons

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Vertebrate ovary

The Vertebrate Ovary: Comparative Biology and Evolution. Edited by R. E. Jones. Pp. 853. (Plenum: New York and London, 1978.) £43.78.

PUBLICATION of another volume (over 800 pages) on the ovary highlights the expansion of research in this aspect of reproductive biology. Consisting of 24 reviews by leading scientists predominantly from the USA the work is mostly comparative in nature. This is exemplified at the beginning and end of the book by chapters on the origin and segregation of primordial germ cells, in which the anuran germ line receives special attention, and on the evolution of the vertebrate ovary. Morphological differentiation of the ovary is also considered from a comparative viewpoint, but biochemical and genetic factors that influence gonadal differentiation are treated only briefly.

The follicle is reviewed extensively: its genesis, morphology and endocrine function; the hormonal control of its growth and maturation; and the production of follicular fluid. The non-atretic follicle is seen as developing in a predominantly oestrogenic micro-environment that influences follicular growth; processes that result in atresia, and the repercussion of this phenomenon on ovarian function, are examined separately.

Understandably, formation of oögonia, maturation of the oocyte and ovulation receive much attention. Most contributors dealing with mechanistic aspects of ovarian physiology summarise their ideas in the form of hypothesis. This is seen in the description of ovulation, and of oocyte maturation in amphibians, in which the elaborate biochemical events induced by steroids that lead to the formation of the meiotic spindle are examined. Work on this latter aspect contrasts with experiments in mammals which have focused rather on the maturation-inducing action of luteinising hormone and on how the resumption of meiosis is prevented by granulosa cells and follicular fluid inhibitor.

Compared with its close contemporary, *The Ovary* (edited by Lord Zuckerman and B. J. Weir; second edition; Academic: London and New York; three volumes; total price £77.70), it is inevitable that this new book contains chapters on similar topics. However, interpretations by different experts frequently result in essays that are complementary rather than merely repetitious. Valuable additions include reviews of ovarian vasculature, with a description of techniques of blood flow measurement and of the effect of

humoral agents, ovarian innervation, and the fascinating fields of follicular selection and vertebrate fecundity. Discussion of the fate of the ruptured follicle is less satisfactory, as reference to the evolutionary significance of the corpus luteum and the comparative aspects of its structure and function are found dispersed among several reviews. The lack of chapters on ovarian pathology and the influence of external factors on ovarian function is attributed to the restrictions of space, but for a book of this quality the reproduction of photomicrographs deserves a better standard.

A selected sentence from a paper

Climatic impact on organic evolution

Climate and Evolution. By Ronald Pearson. Pp. 274. (Academic: London, New York and San Francisco, 1979.) £14.

THIS is an ambitious work that must be taken seriously by anyone interested in palaeoclimatology and biostratigraphy. Such a study, in order to fulfil its title, requires a multi-disciplinary approach to a vast literature and is more than an ordinary mortal could achieve. Pearson, however, is mortal enough, so it is worth seeing where he succeeds and where the book falls short.

The book is organised so that chapters 1-5 are general and 6-11 historical. The introduction concerns evolution only and is brief but to the point. Chapters 2, "A brief history of historical climatology", and 3, "More recent knowledge of climatic change", review the principal hypotheses for climatic change but primarily for changes evident in Quaternary history. This is to some extent balanced by chapter 5, "Long term considerations", with chapter 4, "Geomagnetic considerations", thrown in for good measure. The result is a very brief resume of the mechanisms for climatic change abstracted from many leading authorities in these fields. The remaining chapters are chronological: (6) Palaeozoic; (7) Mesozoic; (8) Tertiary; (9) Quaternary; (10) Late Weichselian and Flandrian; and (11) Climate and [human] history. A consolidated list of more than 750 references and a fairly good index, complete the book.

The author's own viewpoint is evident in the balance of the work. His own publications listed concern Quaternary Coleoptera; and the whole discus-

by Bern (1972) reflects the editor's bias: "we comparative biologists have an aim—the aim to reconstruct from extant species a picture of the evolution of the system in which we are interested". The picture proves complex, frequently displaying diversity rather than conformity. Nonetheless the book should prove valuable for the research scientist, clinician and teacher; and the nature of unanswered questions should stimulate enquiry.

R. B. Heap

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sion is Quaternary-orientated, so that earlier and possibly more radical climatic and evolutionary changes are increasingly obscured through the mists of time. Precambrian history is accorded token mention and the first metazoan (Ediacaran) fauna (surely of prime significance in such a study) receives only a passing reference without definite mention. In the same chapter discussion of climatic change on the megascale is minimal. For example, the role of atmospheric CO₂ is hardly discussed and the possibility of the first half of the Earth's history being climatically hotter than later because of this and other factors, is not considered. Inevitably too, the consideration of Pre-Quaternary history depends on more stratigraphical knowledge than is easy to acquire for the purposes of such a work.

An alternative approach might have been more satisfactory, namely, to review organic evolution and first identify the developments that need explanation and then to compare climatic and other mechanisms in each case against a critical assessment of the chronological precision of the data brought to bear. And here is a difficulty: inevitably the many chronometric age estimates quoted tend to be taken at their numerical face value, and it would require much research to do otherwise.

In conclusion, this work, except for parts of the Quaternary record, does not provide a critical assessment of the climatic impact on organic evolution but it does a most valuable service in bringing together and abstracting a variety of relevant literature and the wide range of ideas necessary as a preliminary. Most specialists will find something here to challenge their thinking.

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obituary

Sir Edward Salisbury

SIR EDWARD JAMES SALISBURY, CBE, FRS, who died on 10 November 1978 at the age of 92, was one of the best known British botanists of his generation.

He was born on 16 April 1886 at Limbrick Hall, Hertfordshire, his father, J. Wright Salisbury, being a member of a distinguished family long resident in the area. He was educated at University College School and then at University College, London, which he entered as an undergraduate in 1905, graduating with an honours degree in botany in 1908. He stayed on as a research student, from 1910 onwards as Quain Student; moved to East London College in 1914 as senior lecturer in botany, and returned to University College as lecturer in 1918. In 1924 he was made university reader in plant ecology, and succeeded F. W. Oliver as Quain Professor in 1929, retaining this post until he was appointed director of the Royal Botanic Gardens, Kew, in 1943. He retired from the directorship in 1956 at the age of 70. He was elected Fellow of the Royal Society in 1933, was awarded its Royal Medal in 1945, and served as Biological Secretary from 1945 until 1955. He was made CBE in 1939 and was knighted in 1946.

Salisbury showed an interest in plants at quite an early age. By his 15th birthday, when one of his presents was a copy of Hooker's *Student's Flora*, he had a garden plot in which wild plants he had collected were labelled with their Latin names, had formed a private herbarium and could identify most of the flowering plants of Hertfordshire. He went to University College during an important period of change in attitude towards the scientific study of plants.

The professor of botany was F. W. Oliver, and A. G. Tansley was a lecturer on his staff from 1893 until his return to Cambridge in 1907. Both had been much influenced in their research and teaching by the striking advances in palaeobotany of the few previous decades. Just before the turn of the century, however, Oliver embarked on his studies of coastal vegetation and Tansley became deeply interested in the 'ecological' and physiological aspects of plant geography as expounded by Warming and Schimper respectively. They had been increasingly dissatis-

fied with the almost exclusive attention to comparative morphology and anatomy which still characterized much botanical teaching and welcomed the ecological emphasis on the plant as a functional whole.

Salisbury was immediately attracted by Oliver's lectures and especially by his field excursions to the north Norfolk coast and elsewhere. After graduation he elected to work with Oliver and two joint papers appeared in 1913, one being a lengthy general account of the ecology of Blakeney Point. Meanwhile his ecological interests were extending to problems of inland vegetation and especially of woodlands, and his now classic studies of variations in the woodland light climate with season and with stage in the coppice-cycle, and their effects on the ground flora, soon attracted attention. He was already laying the foundations of his reputation as a leading academic botanist, and his appointment to the Quain Chair in 1924 greatly increased his influence.

This was early exerted through the series of text-books written in collaboration with F. E. Fritsch. *An Introduction to the Study of Plants* was published in 1914 by G. Bell & Sons and was immediately successful in encouraging the consideration of plants as living and functioning wholes rather than as assemblages of organs, tissues and cells. The last of the famous 'Fritsch & Salisbury' series was *Plant Form and Function* (1938).

While still a research student Salisbury was invited to join the British Vegetation Committee, set up in 1904 to coordinate current work on the survey and study of British vegetation and converted in 1913 into the British Ecological Society. Salisbury was a founder member of the Society and was invited to join its council and to become hon. secretary in 1915, an office he held until 1931. He was elected president for the two years 1929-30.

His presidential address, 'The biological equipment of species in relation to competition' (1929), reveals very clearly how far British ecology, with its primary aim of understanding why plants of some species but not of others grow in a given area, had come to differ from continental ecology with its largely floristic interest and its emphasis on the description and classification of plant communities. Certainly Salisbury was relatively little interested in plant communities as such. His main

concern throughout his life was with those features of individual plant species that were most relevant to survival in a given environment and against given competitors, and in particular those that could readily be assessed quantitatively. The best of his books—*The Reproductive Capacity of Plants* (1942) and *Weeds and Aliens* (1961)—and large numbers of papers in scientific journals, examine the survival-value of features of seed-production, or of vegetation multiplication and spread, in species of different habitats. They are packed with original data, especially of counts and weights of seeds produced by various species in various circumstances. Eleven of the fifteen papers published during the final ten years of his life are of this kind and the last of all, appearing in the year of his death at 92, included a table of estimated annual production of seeds by 49 different weed species 'based upon my own observations from random samples.'

It is relevant to reflect that Professor J. L. Harper's important book, *The Reproductive Biology of Plants*, is in many ways a continuation of *The Reproductive Capacity of Plants*, with a successful elucidation of many points left unexplained by Salisbury but with a considerable widening of scope and a far greater emphasis on experimentation. Characteristically, however, Salisbury had perceived many of what were still the outstanding problems in 1977.

Early in his career Salisbury became a member of the Royal Horticultural Society and was for many years a vice-president. He took great delight in gardening and after his marriage developed a notably attractive and botanically very interesting garden at his home Willow Pool, Radlett. *The Living Garden, or The How and Why of Garden Life*, a skilful popular exposition of the scientific basis of horticulture, appeared in 1945. It was immensely successful and led to the award of the Veitch Memorial Gold Medal of the RHS that same year, and he later received the society's Victoria Medal of Honour. His appointment to Kew was a fitting climax to his career.

Clear-headed, self-confident, determined and articulate, Salisbury was very much a committee man and served on innumerable councils, committees, delegacies and visiting groups. He was governor of several colleges and research institutions and a successful

chairman of many important public bodies. He was short of stature, lively and friendly in manner, but perhaps rather too fond of indulging his delight in lengthy exposition. He was an able administrator. In the later stages of his career, however, he spent much time on his outside commitments.

In 1917 Salisbury married Mabel Elwin-Coles, who died in 1956 after a long illness. There were no children.

A. R. Clapham

C. T. Rajagopal

PROFESSOR Cadambathur Tiruvenkatacharya Rajagopal, a mathematician of considerable standing in India, and solely responsible for the survival in Madras of the Ramanujan Institute, died on 25 April 1978. Even more, perhaps, than his numerous original contributions to mathematics and its history (which were substantial by any standards), it is his devotion to the cause of mathematics in India and to the survival of the Institute (which he served in various capacities from 1951 to 1971) which made him a unique figure in an important period of the history of Indian science, deserving to be remembered by future generations of Indian scientists.

C. T. Rajagopal was born on 8 September 1903. His father, Cadambathur Tiruvenkatacharya, was in the judicial service of the Madras Presidency (now Tamil Nadu). His early education was in Madras; he took his Master's degree in the Madras Presidency College (where many of the most distinguished scientists of India, including Sir C. V. Raman, have been students). There he soon came under the influence of Professor K. Ananda Rau, himself a mathematician of great distinction, who had been G. H. Hardy's student and Ramanujan's contemporary in Cambridge. Ananda Rau is well known and remembered for his valuable contributions to the theory of Tauberian theorems, function-theory and the theory of Dirichlet series; and his tastes and interests were decisive for the orientation of Rajagopal's future scientific career.

After graduating from the Presidency College, Rajagopal joined the Madras Christian College as a lecturer. In 1951, T. Vijayaraghavan, on his appointment as director of the Ramanujan Institute, invited him to join its faculty; the story of Rajagopal coincides with that of the Institute for the following twenty-five years.

The Ramanujan Institute was founded in 1951 as a private institution

by the late Sir Alagappa Chettiar, a noted philanthropist of South India, as 'a small remembrance of a great man (Srinivasa Ramanujan). Its first director, T. Vijayaraghavan, was perhaps the most talented among G. H. Hardy's former students; he died at a comparatively early age in 1955; Rajagopal took over the directorship from him. Already at that time the financial status of the Institute seemed shaky, since Alagappa Chettiar's fortune was melting away; T. Vijayaraghavan's family was left unprovided for, and an appeal to the Prime Minister (the late Jawaharlal Nehru) had to be made in order to rescue them from utter poverty.

In April 1957, when Alagappa Chettiar died, the fate of the Institute hung in the balance; Rajagopal wrote to one of us (S.C.) that the Institute 'will cease to exist on the first of next month,' whereupon the addressee wrote to the Prime Minister, explaining the origin of the Institute and the seriousness of its condition. Nehru's prompt answer was refreshing: 'Even if you had not put in your strong recommendation in favour of the Ramanujan Institute of Mathematics, I would not have liked anything to happen which put an end to it. Now that you have also written to me on this subject, I shall keep in touch with this matter and I think I can assure you that the Institute will be carried on.'

And it was; but haltingly and precariously for the next twelve years. The responsibility for the Institute was divided between the U.G.C. (the federal University Grants Commission) and a reluctant University of Madras. There is no doubt that the Institute would not have survived had it not been for Rajagopal's continuing year after year with an uncertain appointment and often as the sole 'permanent' member of the Institute. In 1963 the future of the Institute and Rajagopal's own means of survival were so much in doubt that he wrote us in the following terms: 'In twenty-one years of service as a teacher (of which the first year was spent in Annamalai University and the rest in Madras Christian College), my salary rose from Rs.100 p.m. to about Rs.240 p.m. and earned for me a provident fund of nearly Rs.8,000. In the next twelve years of my service, in the Ramanujan Institute, my salary scale was Rs.500 - 50 - 800 until I was made a professor with effect from 1st March 1962 on Rs.850 p.m. in the present Madras University scale of Rs.800 - 50 - 1,250. However, my service in the Institute has left me with no savings and no retirement benefits. Thus, after a professional life of thirty and odd years, I find myself without the means to live in complete independence . . . '.

But Rajagopal did continue to serve the Institute for the following six years, and at long last, in August 1967, the Ramanujan Institute was finally adopted by the University of Madras, and in July 1969 a new director was appointed. Its subsequent fortunes do not concern us here; but this left Rajagopal with no pension; not a single *naya paisa* (the new half-penny), as he himself wrote; the undersigned, singly or jointly, must have written dozens of letters to various authorities during the years 1963-1978 to secure for him a modest stipend of Rs.500 p.m.

Despite such administrative and personal worries and frustrations, and without ever an opportunity for visiting any centre of mathematics in Europe or the United States, Rajagopal maintained an unabated output of competent, worthwhile mathematical research, well appreciated by co-workers in his favourite fields, chiefly Tauberian theorems and entire functions. In the latter part of his life, he became actively interested in the history of medieval Indian mathematics, to which he contributed a number of important papers, partly in collaboration with others; the last one, a joint article with M. S. Rangachari, appeared only a few weeks before Rajagopal's death, in C. Truesdell's well-known *Archive for History of Exact Sciences*, vol. 18, pp. 89-102, 1978. There it is shown that a number of power-series expansions for trigonometric and inverse trigonometric functions, discovered in the 17th century by Gregory, Newton and Leibniz (and independently, about the same time, by the Japanese Seki), had been known to Kerala mathematicians more than a century before.

Professor C. T. Rajagopal is survived by his wife, Mrs. Rukmini Rajagopal (now living in straitened circumstances, it must be pointed out, due to the lack of provision for a pension fund at the Ramanujan Institute). Tragically, Rajagopal's death occurred just as a grant had been approved to support the continuation of his and M. S. Rangachari's historical investigations, which were to appear eventually as a monograph on Kerala mathematics.

India may well have produced mathematicians of greater versatility and depth than C. T. Rajagopal, and will, one hopes, produce more such in the future; but none has served the cause of mathematics more selflessly nor with greater devotion. As both of us happen to have been personally associated, for many years, with this modest and talented man, we find it fitting that his long years of service be placed on record.

S. Chandrasekhar
André Weil